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Supplementary information

Survival and biomarker analyses from the OpACIN-neo and OpACIN neoadjuvant immunotherapy trials in stage III melanoma

In the format provided by the authors and unedited

SUPPLEMENTARY INFORMATION

Supplement to: 'Survival and Biomarker Analyses from the OpACIN-neo and OpACIN Neoadjuvant Immunotherapy Trials in Stage III Melanoma **'**

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Supplementary Table 1: Baseline Characteristics OpACIN-neo

	Arm A: 2xI3N1 (n=30)	Arm B: 2xI1N3 (n=30)	Arm C: 2xI3>2xN3 (n=26)
Age			
Years, median (range)	63.5 (18-79)	54 (31-74)	58.5 (27-80)
<60, n (%)	13 (43)	21 (70)	18 (69)
Sex, n (%)			
Male	19 (63)	14 (47)	16 (62)
Female	11 (37)	16 (53)	10 (38)
Geographic region, n (%)			
Europe	16 (53)	18 (60)	14 (54)
Australia	14 (47)	12 (40)	12 (46)
ECOG performance status, n (%)			
0	30 (100)	29 (97)	26 (100)
1	-	1 (3)	-
Primary tumor, n (%)			
T1-T2	13 (43)	15 (50)	10 (38)
T3-T4	9 (30)	6 (20)	9 (35)
Unknown primary	8 (27)	9 (30)	7 (27)
Ulceration of primary tumor, n (%)			
Yes	8 (27)	7 (23)	6 (23)
No	14 (47)	13 (43)	12 (46)
Unknown	8 (27)	10 (33)	8 (31)
Sum of diameter target lesions median (range)	23 (15-70)	25 (15-61)	24 (15-64)
Location affected lymph node, n (%)			
Neck	5 (17)	3 (10)	6 (23)
Axilla	13 (43)	17 (57)	14 (54)
Axilla and Neck	3 (10)	-	-
Groin	9 (30)	9 (30)	6 (23)
Other	-	1 (3)	-
LDH, n (%)			
<ULN	30 (100)	30 (100)	25 (96)
>ULN	-	-	1 (4)
PD-L1 expression on tumor cells, n (%)			
<1%	13 (43)	12 (40)	16 (62)
1-50%	9 (30)	6 (20)	4 (15)
>50%	1 (3)	4 (13)	2 (8)
Missing	7 (23)	8 (27)	4 (15)

Clinical baseline characteristics of all patients (=86) separated by treatment arm. Percentages may not total 100 because of rounding. PD-L1 immunohistochemistry was performed using with an automated laboratory-validated immunohistochemistry assay, with the use of the 22C3 antibody on a Ventana platform (BenchMark Ultra autostainer, Ventana Medical Systems, Tuscon, AZ, USA).

Supplementary Table 2: Overview of first EFS event

Event	Arm A	Arm B	Arm C
Any (%)	**3 (10)**	**7 (23)**	**5 (19)**
Local PD precluding definitive surgery	-	-	-
Distant PD precluding definitive surgery	-	1 (3)	1 (4)
Local recurrence	-	2 (7)	-
Distant recurrence	2 (7)	4 (13)	4 (15)
Death due to toxicity	1 (3)[1]	-	-
Death due to other causes	-	-	-

Overview of the first event per patient separated by treatment arm. [1] One patient died due to a late-onset immune-related encephalitis.

Supplementary Table 3: Subsequent therapies

	OpACIN-neo			OpACIN	
	Arm A (N=30)	Arm B (N=30)	Arm C (N=26)	Adjuvant (n=10)	Neoadjuvant (n=10)
Any therapy	**3 (10)**	**7 (23)**	**6 (23)**	**4 (40)**	**2 (20)**
Adjuvant radiotherapy	2 (7)		1 (4)		
Surgery	1 (3)	5 (17)	2 (8)	2 (20)	1 (10)
Radiotherapy	1 (3)	2 (7)	2 (8)	3 (30)	
T-VEC					
Anti-PD1		2 (7)	1 (4)	1 (1)	
Ipilimumab + nivolumab	1 (3)				
BRAFi + MEKi	1 (3)	5 (17)	4 (15)	4 (4)	1 (10)
Clinical trial	1 (3)		2 (8)	1 (10)	

Patients could have had more than one type of therapy. Data regarding subsequent therapy after relapse were only available if it was entered in the database at the time of data cut-off. Data are presented as n (%).

Supplementary Table 4: Immunotherapy related adverse events

	Arm A		Arm B		Arm C		Total	
Event	**All grade**	**Grade 3-5**	**All grade**	**Grade 3-5**	**All grade**	**Grade 3-5**	**All grade**	**Grade 3-5**
Any event	**30 (100%)**	**13 (43%)**	**30 (100%)**	**8 (27%)**	**26 (100%)**	**14 (54%)**	**86 (100%)**	**35 (41%)**
Fatigue	20 (67%)		17 (57%)		14 (54%)		51 (59%)	
Rash	18 (60%)	2 (7%)	12 (40%)	1 (3%)	20 (77%)	3 (12%)	50 (58%)	6 (7%)
Pruritus	12 (40%)		12 (40%)		11 (42%)		35 (41%)	
AST increased	12 (40%)	5 (17%)	6 (20%)	1 (3%)	11 (42%)	1 (4%)	29 (34%)	7 (8%)
Vitiligo	13 (43%)		7 (23%)		9 (35%)		29 (34%)	
ALT increased	12 (40%)	6 (20%)	6 (20%)	1 (3%)	10 (38%)	2 (8%)	28 (33%)	9 (10%)
Diarrhea	7 (23%)	1 3%)	5 (17%)	1 (3%)	11 (42%)	3 (12%)	23 (27%)	5 (6%)
Hyperthyroidism	12 (40%)		2 (7%)		9 (35%)	1 (4%)	23 (27%)	1 (1%)
Headache	8 (27%)	1 (3%)	5 (17%)		4 (15%)		17 (20%)	1 (1%)
Hypothyroidism	6 (20%)		5 (17%)		5 (19%)		16 (19%)	
Dry mouth	6 (20%)		4 (13%)		5 (19%)		15 (17%)	
Fever	4 (13%)		4 (13%)	1 (3%)	7 (27%)		15 (17%)	1 (1%)
Arthralgia	4 (13%)		5 (17%)		3 (12%)		12 (14%)	
Colitis	3 (10%)	2 (7%)	1 (3%)		7 (27%)	5 (19%)	11 (13%)	7 (8%)
Nausea	4 (13%)		1 (3%)		6 (23%)	1 (4%)	11 (13%)	1 (1%)
Adrenal insufficiency	3 (10%)	1 (3%)	2 (7%)		4 (15%)	2 (8%)	9 (10%)	3 (3%)
Dry eye	3 (10%)		3 (10%)		3 (12%)		9 (10%)	
Flu like symptoms	1 (3%)		5 (17%)		2 (8%)		8 (9%)	
Ggt increased	4 (13%)	2 (7%)	4 (13%)	3 (10%)			8 (9%)	5 (6%)
Abdominal pain	3 (10%)		1 (3%)		3 (12%)		7 (8%)	
Infusion related reaction	(0%)		5 (17%)		2 (8%)		7 (8%)	
Anemia	2 (7%)		1 (3%)		3 (12%)		6 (7%)	
Malaise	3 (10%)		3 (10%)				6 (7%)	
Pneumonitis	2 (7%)		1 (3%)		3 (12%)		6 (7%)	
Serum amylase increased	3 (10%)	1 (3%)	2 (7%)	1 (3%)	1 (4%)		6 (7%)	2 (2%)
ALP increased	2 (7%)		3 (10%)		0		5 (6%)	
Anorexia	1 (3%)		2 (7%)		2 (8%)		5 (6%)	
Concentration impairment	2 (7%)		2 (7%)		1 (4%)		5 (6%)	
Cough	1 (3%)		3 (10%)		1 (4%)		5 (6%)	
Dry skin	2 (7%)		1 (3%)		2 (8%)		5 (6%)	
Dysgeusia	1 (3%)		1 (3%)		3 (12%)		5 (6%)	
Dyspnea	2 (7%)		2 (7%)		1 (4%)		5 (6%)	
Lipase increased	2 (7%)	1 (3%)	2 (7%)		1 (4%)		5 (6%)	1 (1%)
Sarcoid like reaction			3 (10%)		2 (8%)		5 (6%)	
Back pain			3 (10%)		1 (4%)		4 (5%)	

Gastritis	1 (3%)		1 (3%)		2 (8%)	1 (4%)	4 (5%)	1 (1%)
Hoarseness	1 (3%)		2 (7%)		1 (4%)		4 (5%)	
Myalgia	1 (3%)		2 (7%)		1 (4%)		4 (5%)	
Weight loss	2 (7%)				2 (8%)		4 (5%)	
Alopecia			1 (3%)		2 (8%)		3 (3%)	
Blurred vision	2 (7%)		1 (3%)				3 (3%)	
Hyponatremia			2 (7%)	1 (3%)	1 (4%)	1 (4%)	3 (3%)	2 (2%)
Sinusitis			3 (10%)				3 (3%)	
Stomach pain			1 (3%)		2 (8%)	1 (4%)	3 (3%)	1 (1%)
Vomiting	2 (7%)				1 (4%)		3 (3%)	
Creatinine increased	1 (3%)		1 (3%)				2 (2%)	
Floaters	1 (3%)		1 (3%)				2 (2%)	
Hypophysitis	1 (3%)		1 (3%)				2 (2%)	
Lymph node pain			1 (3%)		1 (4%)		2 (2%)	
Radiculitis			1 (3%)		1 (4%)	1 (4%)	2 (2%)	1 (1%)
Tendinitis	2 (7%)						2 (2%)	
Uveitis			1 (3%)	1 3%)	1 (4%)		2 (2%)	1 (1%)
Acidosis					1 (4%)	1 (4%)	1 (1%)	1 (1%)
Acute kidney injury			1 (3%)				1 (1%)	
Allergic rhinitis					1 (4%)		1 (1%)	
Arthritis					1 (4%)		1 (1%)	
Ataxia					1 (4%)	1 (4%)	1 (1%)	1 (1%)
Atrial fibrillation					1 (4%)		1 (1%)	
Blood bilirubin increased	1 (3%)	1 (3%)					1 (1%)	1 (1%)
Chest pain-cardiac					1 (4%)		1 (1%)	
Chills					1 (4%)		1 (1%)	
Conjuctivitis					1 (4%)		1 (1%)	
Constipation	1 (3%)						1 (1%)	
Edema face			1 (3%)				1 (1%)	
Edema neck			1 (3%)				1 (1%)	
Encephalitis							1 (1%)	1 (1%)
Esophageal obstruction					1 (4%)		1 (1%)	
Eye pain	1 (3%)						1 (1%)	
Flank pain			1 (3%)				1 (1%)	
Flashing lights					1 (4%)		1 (1%)	
Flatulence					1 (4%)		1 (1%)	
Gastoparesis					1 (4%)		1 (1%)	
Gastroesophageal reflux disease			1 (3%)				1 (1%)	
Glucose intolerance					1 (4%)	1 (4%)	1 (1%)	1 (1%)

Hepatitis	1 (3%)	1 (3%)					1 (1%)	1 (1%)
Hot flashes					1 (4%)		1 (1%)	
Hypokalemia					1 (4%)	1 (4%)	1 (1%)	1 (1%)
Hyperglycemia			1 (3%)				1 (1%)	
Hypotension	1 (3%)	1 (3%)					1 (1%)	1 (1%)
Infusion site extravasation							1 (1%)	
Lethargy			1 (3%)				1 (1%)	
Meningitis			1 (3%)	1 (3%)			1 (1%)	1 (1%)
Nail ridging			1 (3%)				1 (1%)	
Neutrophil count decreased	1 (3%)						1 (1%)	
Oral pain	1 (3%)						1 (1%)	
Paresthesia			1 (3%)				1 (1%)	
Peripheral motor neuropathy					1 (4%)	1 (4%)	1 (1%)	1 (1%)
Platelet count decreased	1 (3%)						1 (1%)	
Psoriasis					1 (4%)		1 (1%)	
Restless legs syndrome	1 (3%)						1 (1%)	
Salivary duct inflammation			1 (3%)				1 (1%)	
Sinus pain					1 (4%)		1 (1%)	
Sinus tachycardia	1 (3%)						1 (1%)	
Skin infection					1 (4%)		1 (1%)	
Urinary retention	1 (3%)						1 (1%)	

Information on all immunotherapy-related adverse events of all grade and all grade 3-5 adverse events, both for the total treatment population and per treatment arm.

Supplementary Table 5: Ongoing immunotherapy and surgery related adverse events

Immune-related adverse event	Grade 1-2	Grade 3-5	Total
Any event	**53 (65%)**	**2 (3%)**	**55 (68%)**
Skin hypopigmentation	28 (35%)		28 (35%)
Fatigue	12 (15%)		12 (15%)
Hypothyroidism	11 (14%)		11 (14%)
Rash	9 (11%)		9 (11%)
Adrenal insufficiency	8 (10%)		8 (10%)
Dry mouth	8 (10%)		8 (10%)
Pruritus	7 (9%)		7 (9%)
Arthralgia	6 (7%)		6 (7%)
Dry eye	3 (4%)		3 (4%)
Concentration impairment	2 (2%)		2 (2%)
Creatinin increased	2 (2%)		2 (2%)
Dyspnea	2 (2%)		2 (2%)
Alopecia	1 (1%)		1 (1%)
Atrial fibrillation	1 (1%)		1 (1%)
Restless legs syndrome	1 (1%)		1 (1%)
Peripheral motor neuropathy		1 (1%)	1 (1%)
Sarcoid like reaction	1 (1%)		1 (1%)
Sinus pain	1 (1%)		1 (1%)
Uveitis		1 (1%)	1 (1%)
Weight loss	1 (1%)		1 (1%)
Dysphagia	1 (1%)		1 (1%)
Surgery-related adverse event	**Grade 1-2**	**Grade 3-5**	**Total**
Any event	**31 (38%)**		**31 (38%)**
Lymphedema	17 (21%)		17 (21%)
Seroma	10 (12%)		10 (12%)
Pain	6 (7%)		6 (7%)
Paraesthesia	5 (6%)		5 (6%)
Joint range of motion decreased	1 (1%)		1 (1%)

All ongoing immunotherapy-related adverse events and surgery-related adverse events of all grade, grade 1-2 and grade 3-5. All patients that were still alive at time of data cut-off were included (n=81).

Supplementary Table 6: Surgery related adverse events

	Arm A		Arm B		Arm C		Total	
Event	**All grade**	**Gr 3-5**	**All grade**	**Gr 3-5**	**All grade**	**Gr 3-5**	**All grade**	**Gr 3-5**
Any event	**25 (83%)**	**4 (13%)**	**25 (83%)**	**4 (13%)**	**20 (77%)**	**4 (15%)**	**70 (81%)**	12 **(14%)**
Seroma	16 (53%)		15 (50%)		10 (38%)		41 (48%)	
Wound infection	14 (47%)	4 (13%)	10 (33%)	2 (7%)	10 (38%)	3 (12%)	34 (40%)	9 (10%)
Pain	5 (17%)		5 (17%)		3 (12%)		13 (15%)	
Lymphedema	5 (17%)		5 (17%)		2 (8%)		12 (14%)	
Edema limb	3 (10%)		5 (17%)		1 (4%)		9 (10%)	
Wound dehiscence	2 (7%)		3 (10%)		4 (15%)		9 (10%)	
Paresthesia	1 (3%)		1 (3%)		4 (15%)		6 (7%)	
Fever	2 (7%)		1 (3%)		2 (8%)		5 (6%)	
Joint range of motion decreased	1 (3%)		1 (3%)				2 (2%)	
Rash	2 (7%)						2 (2%)	
Skin infection	1 (3%)		1 (3%)				2 (2%)	
Acute kidney injury	1 (3%)						1 (1%)	
Alopecia			1 (3%)				1 (1%)	
Anaemia					1 (4%)		1 (1%)	
Atrial fibrillation	1 (3%)						1 (1%)	
Chills	1 (3%)						1 (1%)	
Colitis					1 (4%)		1 (1%)	
Concentration impairment					1 (4%)		1 (1%)	
Drain leakage			1 (3%)				1 (1%)	
Dysphagia	1 (3%)						1 (1%)	
Dysphonia			1 (3%)				1 (1%)	
Fatigue					1 (4%)		1 (1%)	
Haedache			1 (3%)				1 (1%)	
Hematoma	1 (3%)						1 (1%)	
Chyle leakage due to surgery			1 (3%)	1 (3%)			1 (1%)	1 (1%)
Surgical site swelling						1 (4%)	1 (1%)	1 (1%)
Intraoperative haemorrhage			1 (3%)	1 (3%)			1 (1%)	1 (1%)
Nausea	1 (3%)						1 (1%)	
Postoperative hemorrhage					1 (4%)	1 (4%)	1 (1%)	1 (1%)
Serum amylase increased					1 (4%)		1 (1%)	
Thrombosis	1 (3%)						1 (1%)	

Information on all surgery-related adverse events of grade 1-2 and all grade 3-5 adverse events, both for the total treatment population and per treatment arm.

Supplementary Table 7: Univariable and multivariable analysis for pathologic response

	Univariable analysis			Multivariable analysis *		
	OR	**(95% CI)**	**P-value**	**OR**	**(95% CI)**	**P-value**
Continent						
Australia vs Europe	2.500	(0.89-7.76)	0.092	1.644	(0.33-9.35)	0.550
Gender						
Male vs Female	2.897	(1.06-8.32)	0.041**	1.666	(0.39-7.27)	0.488
Age						
Continuous (per year)	1.059	(1.02-1.10)	0.003**	1.007	(0.95-1.07)	0.795
IFN-γ gene expression						
z-score	2.11	(1.11-4.33)	0.028**	3.763	(1.57-11.08)	0.007**
TMB						
Log(TMB)	7.97	(1.81-49.29)	0.012**	14.194	(1.85-182.18)	0.021**

** Performed for the 59 patients for whom IFN-γ and TMB could be analyzed*
*** Statistically significant*

Association between different baseline / tumor characteristics and pathologic response calculated via univariable and multivariable analysis. P-values are calculated using the logistic regression method (two-sided).

Supplementary Table 8: Baseline Characteristics per Continent

	Australia (n=38)	Europe (n=48)
Age		
Years, median (range)	60 (31-80)	53 (18-79)
<60, n (%)	17 (44.7)	30 (62.5)
Sex , n (%)		
Male	25 (65.8)	24 (50.0)
Female	13 (34.2)	24 (50.0)
Treatment arm, n (%)		
A	14 (36.8)	16 (33.3)
B	12 (31.6)	18 (37.5)
C	12 (31.6)	14 (29.2)
Primary tumor, n (%)		
T1-T2	15 (39.5)	23 (47.9)
T3-T4	9 (23.7)	15 (31.3)
Unknown primary	14 (36.8)	10 (20.8)
Ulceration of primary tumor, n (%)		
Yes	10 (26.3)	11 (22.9)
No	12 (31.6)	26 (54.2
Unknown	16 (42.1)	11 (22.9)
Sum of diameter target lesions median (range)	24 (14-70)	24 (14-64)
Location affected lymph node, n (%)		
Neck	5 (13.2)	9 (18.8)
Axilla and Neck	2 (5.3)	1 (2.1)
Axilla	19 (50.0)	25 (52.1)
Groin	11 (28.9)	13 (27.1)
Other	1 (2.6)	0 (0.0)
LDH, n (%)		
<ULN	37 (97.4)	48 (100)
>ULN	1 (2.6)	0 (0)
PD-L1 expression on tumor cells, n (%)		
<1%	17 (44.7)	24 (50.0)
1-50%	9 (23.7)	19 (20.8)
>50%	5 (13.2)	2 (4.2)
Missing	7 (18.4)	12 (25.0)

Clinical baseline characteristics of all patients (=86) separated by continent. Percentages may not total 100 because of rounding. PD-L1 immunohistochemistry was performed using with an automated laboratory-validated immunohistochemistry assay, with the use of the 22C3 antibody on a Ventana platform (BenchMark Ultra autostainer, Ventana Medical Systems, Tuscon, AZ, USA).

Supplementary Table 9: Endpoint definitions

Endpoint	Definition
Pathologic response rate	The proportion of patients with <50% viable tumor cells in the tumor bed and underwent definitive surgery. Pathologic response was centrally assessed by two blinded pathologists.
Pathologic complete response	no viable tumor cells in the tumor bed area
Pathologic near-complete response	>0%- ≤10% viable tumor cells in the tumor bed area.
Pathologic partial response	>10% – <50% viable tumor cells in the tumor bed area.
Event-free survival	The time from randomization to the first occurrence of any of the following events: progression of disease that precludes definitive surgery, local or distant recurrence or death due to any cause.
Relapse-free survival	The time from definitive surgery to the first occurrence of any of the following events: local or distant recurrence or death due to any cause.

Pathologic endpoint definitions defined according to the guidelines of the International. Neoadjuvant Melanoma Consortium (INMC).[1]

References

1 Tetzlaff, M. T. *et al.* Pathological assessment of resection specimens after neoadjuvant therapy for metastatic melanoma. *Ann Oncol* **29**, 1861-1868, doi:10.1093/annonc/mdy226 (2018).

Multicenter Phase 2 Study to Identify the Optimal neo-Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN-neo)

Version 1/ 17 October 2016

PROTOCOL TITLE

Multicenter Phase 2 Study to Identify the Optimal neo-Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN-neo)

Protocol ID	**CA209-701/M16OPN/NL58906.031.16**
Short title	**Optimal Neo-adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN-neo)**
EudraCT number	***2016-001984-35***
Version	**1**
Date	**17 October 2016**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
AR	**Adverse Reaction**
BL	**Baseline**
BMS	**Bristol Myers Squibb**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CTLA-4	**cytotoxic T-lymphocyte-associated protein 4**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
irAE	**Immune related adverse event**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
PD-1	**Programmed death receptor-1**
PBMC	**Peripheral Blood Mononuclear Cells**
pPR	**Pathological partial response**
pCR	**Pathological complete response**
RECIST	**Response Evaluation Criteria In Solid Tumours**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a**

study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.

SUSAR **Suspected Unexpected Serious Adverse Reaction**

Wbp **Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**

WMO **Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: T cell checkpoint blockade by anti-CTLA and/or anti-PD1 is currently the most promising therapy in late stage melanoma to induce long-term benefit or even cure. Particularly the combination of ipilimumab and nivolumab induces high response rates and promising response depth. This raises the question whether ipilimumab and/or nivolumab could also become standard therapy in adjuvant treatment of melanoma. Ipilimumab as adjuvant therapy significantly improves recurrence free survival. Recently overall survival data were published and demonstrated a significant overall survival benefit for patients in the ipilimumab group with a 5 year overall survival rate of 65% compared to 54% in the placebo group. Nivolumab and pembrolizumab have been tested in large phase 3 randomized trials of which the results are expected to be presented not before 2018.

In contrast to chemotherapeutic approaches, immunotherapeutic approaches depend on sufficient activation of the immune system. To become fully activated, T cells require two signals. The first signal is provided by the interaction of the (tumor-) antigen presented in the major histocompatibility complex (MHC) on the antigen-presenting cell (APC) to the T cell receptor (TCR) on the T cell (signal 1). In parallel, a large number of co-inhibitory and co-stimulatory interactions – so-called T cell checkpoints - modulate the outcome of the TCR – pMHC interaction. Antibody-based interference with the T cell checkpoints CTLA-4 and PD-1 has been shown to improve tumor-specific T cell responses and to result in a significant clinical benefit in patients with melanoma and other cancers.

The notion that T cell checkpoint inhibition is of greatest value at the moment of TCR triggering has potentially significant consequences for the use of checkpoint targeting antibodies as adjuvant therapies. Specifically, as the amount of antigen that can provide this signal 1 will correlate with tumor load, adjuvant immunotherapy can be assumed to work most efficiently, when adjuvant treatment is initiated prior to surgery. A phase 1 study testing neo-adjuvant and adjuvant application of 4 courses of ipilimumab + nivolumab in stage III melanoma (OpACIN, CA209-278, NCT02437279) has almost finished patient inclusion. Importantly, data obtained show that neo-adjuvant ipilimumab + nivolumab treatment is feasible, resulting in regression of metastasis of 8/10 (80%) patients evaluated so far. At the same time, (neo)-adjuvant treatment resulted in grade 3/4 toxicities in 16/18 (89%) of the currently evaluable patients and only 2/18 patients received all four courses ipilimumab + nivolumab. In the neo-adjuvant arm 9/10 (90%) patients, and in the adjuvant arm 7/8 (88%) patients developed one of more grade 3 or 4 adverse events.

The observed feasibility and pathological responses, combined with the observed toxicity, form the rationale for the design of the subsequent phase 2 trial, OpACIN-neo, that will compare safety and efficacy of neo-adjuvant ipilimumab + nivolumab at three different

schemes combining ipilimumab and nivolumab. Aim is to identify a combination scheme that maintains the high rate of clinical responses observed, but at lower toxicity.

Objectives:

Primary objective:

- To compare safety (immune related adverse events during the first 12 weeks) of three different neo-adjuvant combination schemes of ipilimumab + nivolumab

- To compare radiological and pathological response rates at week 6 of the three neo-adjuvant combination schemes

Secondary objectives:

- To describe relapse free survival
- To describe rate and type of late adverse events (up to 3 years)
- To determine immune activating capacity of the alternative combination schemes

Study design:

This is an open-label three-arm phase 2 trial (including a Simon stage 2 design) consisting of 90 stage III melanoma patients randomized 1:1:1 to receive either 2 courses 3 mg/kg ipilimumab + 1 mg/kg nivolumab every 3 weeks (Arm A), 2 courses 1 mg/kg ipilimumab + 3 mg/kg nivolumab every 3 weeks (Arm B), or 2 courses ipilimumab 3 mg/kg, directly followed by 2 courses nivolumab 3 mg/kg every 2 weeks (Arm C). All three treatment arms are applied prior to surgery at week 6, and are neo-adjuvant (30 patients per arm, Figure 1). Patients will be stratified according to treatment center. An interim analysis will be performed after 13 patients have been included in each arm (see 4.5), thus in total 39 patients have been included.

Study population:

Resectable stage III melanoma patients with measurable lymph node metastases (according to RECIST 1.1) that can be biopsied, with no history of in-transit metastases within the last 6 months, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, and 18 years or older.

Intervention:

Patients will be treated pre-surgically for 6 weeks with the combination of ipilimumab + nivolumab at three different combination schemes (30 patients per arm, Figure 1).

Medicine tested: 2 courses ipilimumab 3 mg/kg plus nivolumab 1 mg/kg q3wks, versus 2 courses ipilimumab 1 mg/kg plus nivolumab 3 mg/kg q3wks, versus 2 courses ipilimumab 3 mg/kg q3wks, directly followed by 2 courses nivolumab 3 mg/kg every 2 weeks.

Lab testing (incl. collection of PBMC and EDTA blood for plasma and thrombocyte isolation) will be performed during screening, at baseline, week 6 pre-surgery and directly post-surgery, and at week 12.

Tumor biopsies/material preservation is required at baseline and during surgery.

CT scans will be required at baseline and at week 6.

Follow-up will start at week 12 with a CT or PET /CT scan according to the individual center's standard. Subsequent follow-up will be by PET/CT or CT scans according to the national/institutional standards for high-risk melanoma.

Collection of another PBMC and EDTA blood (for plasma and thrombocyte isolation) sample, and collection of tumor biopsies will be performed at the time point of relapse.

Figure 1

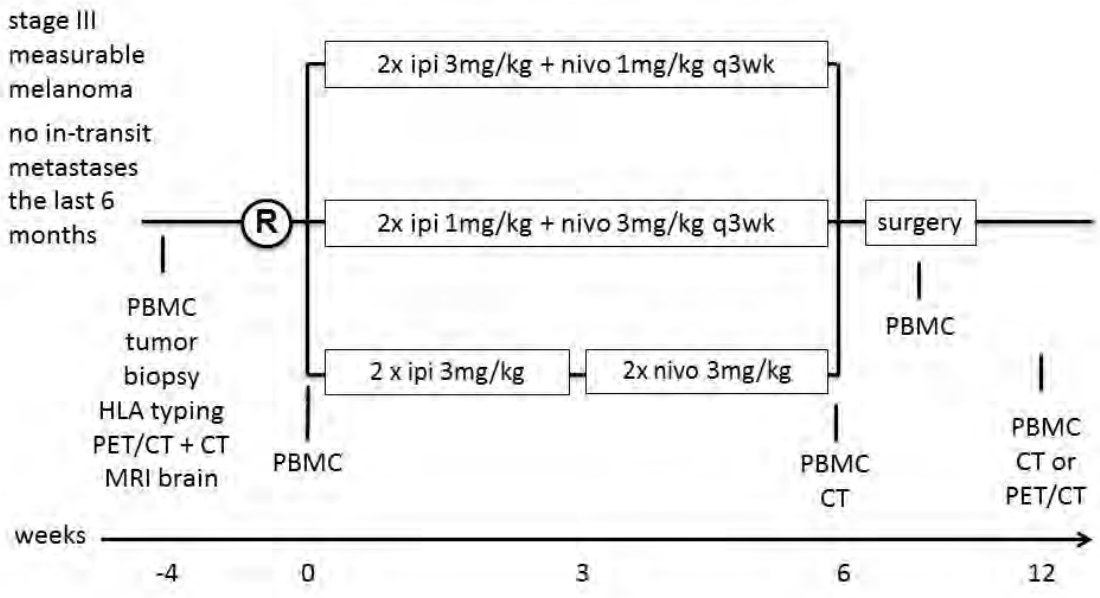

Figure 1: Schematic overview of OpACIN-neo study

Main study parameters/endpoints:

Primary endpoints:

- Safety as measured by the frequency of grade 3/4 immune-related adverse events (during the first 12 weeks).
- Response rate according to RECIST 1.1 at week 6
- Pathological response according to central revision (pathology of NKI).

An interim analysis will be performed after 13 patients have been included in each arm (see 4.5), thus in total 39 patients have been included.

Secondary endpoints:

- Recurrence Free Survival (RFS)
- Description of late adverse event (up to 3 years after treatment initiation) according to CTCAE v4.03
- Description of associations of mutational load, RNA tumor signatures, and tumor educated platelet signatures with tumor immune infiltrates and response
- Alteration in magnitude or breadth of the neo-antigen specific T cell responses in peripheral blood from baseline to surgery at week 6 in each 10 randomly chosen patients per arm.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Until recently, there was no standard adjuvant therapy for patients with high-risk stage III melanoma. Post-surgery adjuvant radiotherapy is commonly applied, because it has been shown to marginally improve local disease control, but neither benefit in RFS, nor overall survival (OS), can be achieved in this high-risk patient population [1, 2]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable survival benefit (two out of three meta-analyses found no OS benefit [3-5]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment. Adjuvant systemic immunotherapy with ipilimumab is the only treatment so far to improve RFS and OS in stage III melanoma [6]. Immune-related grade 3 or 4 adverse events occurred in 41.6% of patients treated with ipilimumab 10 mg/kg adjuvant.

Participants of this study will be exposed to two immunotherapeutic agents (ipilimumab and nivolumab) known to induce immune related adverse events at a high percentage when combined together [7]. This has been also observed in the precursor phase 1 trial (OpACIN), in which, so far, 24 patients have been screened and 18 included into the study (6 screen

failures due to detection of distant metastases during dissemination screening, 18 currently evaluable for toxicity). In this study patients are randomized between either 4 courses ipilimumab+nivolumab adjuvant or 2 courses ipilimumab+nivolumab neo-adjuvant and 2 courses adjuvant. All 10 patients in the neo-adjuvant arm have undergone lymph-node dissection on the planned time-point and no patients experienced grade 3/4 SUSARs attributed to pre-surgery immunotherapy. In that way the trial has reached already its primary feasibility endpoint.

However, 16/18 (89%) developed grade 3/4 treatment-related toxicities, with one additional patient perceiving long-term grade 2 colitis, requiring prednisolon, adding up to currently 17/18 (94%) of patients with clinical relevant immune related adverse events. One patient developed life-threatening Steven-Johnson syndrome (after 3 courses ipilimumab + nivolumab) that recovered upon mycophenolate, one patient developed severe long-term colitis that has been stabilized with tacrolimus and recovered after months, and one patient developed insulin dependent diabetes mellitus. Due to these toxicities all, except 2 patients so far, had to stop earlier resulting in a maximum application of 2-3 courses of ipilimumab + nivolumab, independent of neo-adjuvant versus adjuvant treatment.

Importantly, to date neo-adjuvant application of 2 courses of ipilimumab + nivolumab resulted in profound tumor regression in 8/10 patients (3 pCR, 3 near pCRs with only remaining micro-metastasis and 2 pPR with remaining metastasis of 0.5mm and 9 mm, 1 SD and 1 PD. Two of the patients in the neo-adjuvant arm so far relapsed (interestingly these were the two non-responders, while in the adjuvant arm relapse post-surgery was observed in 3/8 patients.

These observations have led to the design of this phase 2 trial that will test only 2 courses of neo-adjuvant ipilimumab + nivolumab with tighter discontinuation rules (Chapters 6.7 - 6.9), and in 3 different combination schemes with the aim to reduce toxicity while preserving efficacy.

The design of the additional combination schemes to be tested (arm B and C) in this study are based on several observations from other early phase trials in stage 4 melanoma: a) in the phase 1 study testing ipilimumab + nivolumab in late stage melanoma also 4 courses of a lower dose of ipilimumab (1 mg/kg) were tested in combination with nivolumab 3 mg/kg indicating equally efficacy (CA209-004) [8], b) data from the Keynote 029 study testing low dose ipilimumab (1 mg/kg) with the standard dose of pembrolizumab (2 mg/kg) in 153 patients indicate that frequency of grade 3/4 toxicity is lower (to 42%), while disease control is preserved (G. Long, ASCO 2016 meeting), and c) data from a retrospective analysis of late stage melanoma patients treated at the NKI with 2 courses ipilimumab 3 mg/kg q3wks, directly followed the next day by pembrolizumab or nivolumab at standard dosing (2 mg/kg

q3wks or 3 mg/kg q2wks respectively). This sequential scheme also induced a similar disease control rate, as compared to the phase 3 trial testing the standard scheme ipilimumab + nivolumab [9], but was less toxic. We observed in only 38% of the patients grade 3/4 treatment-related toxicity (Meerveld, Rozeman, Blank, et al., submitted to Annals of Oncology). Subsequently, we regularly treated stage 4 melanoma patients with ipilimumab 3mg/kg for 1 or 2 courses, directly followed by nivolumab or pembrolizumab, starting on the same day after the ipilimumab infusion (2 hours observation interval), observing no additional toxicities (manuscript in preparation).

Thus, the risk of neo-adjuvant immune related toxicity in OPACIN-neo is considered to be lower (mainly in arm B or C) than that of patients within the phase I OpACIN trial.

In addition, patients within this trial are not allowed to be treated with local radiotherapy, which bears the risk of reduced local tumor control. Considering the fact that local irradiation only improves local tumor control, but does not improve RFS or OS [1], participation in this trial might offer the chance for improved recurrence free survival. This is based on the observation that adjuvant ipilimumab has already shown RFS benefit in a prospective randomized controlled phase 3 trial in stage III patients, the combination of ipilimumab with nivolumab induces superior response rates as compared to ipilimumab alone in stage IV melanoma [6, 8-10], and the high response rates observed in the neo-adjuvant arm of the OpACIN trial.

1. INTRODUCTION AND RATIONALE

Background Melanoma

Melanoma is one of the fastest growing malignancies with more than 5,000 new diagnoses per year and almost 800 deaths in 2012 in the Netherlands [Dutch tumor registry (IKNL), www.iknl.nl], and approximately 130,000 new diagnoses worldwide, with 37,000 deaths (WHO, 2012). Surgical resection is the treatment of choice for localized melanoma and frequently results in cure for the early stages (stage 1 and 2), with around 90% long-term survival rate for stage 1 melanoma [11]. However, patients with lymph-node involvement (stage 3), including those detected only by sentinel node biopsy, have a high risk of local and distant relapse after surgery, and 5 year survival drops to 39-70% in this patient group [11]. Specifically, patients with stage IIIB disease [macroscopic / palpable (=non-SN detected)] have a five year survival between 20 – 59%, with certain subgroups (number of involved nodes, extracapsular extension, iliac involvement in groin metastases) showing even worse survival rates of around 5% at 5-years [11-13]. Until recently, no clinically significant improvement in survival has to date been achieved by adjuvant therapy in this patient group. Adjuvant radiotherapy has been shown to marginally improve local disease control, but neither benefit in RFS, nor in OS, has been achieved in this high-risk patient group [1, 2]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable OS benefit (two out of three meta-analyses found no OS benefit [3-5]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe, and is, for example, not applied in The Netherlands.

Current standard treatment in melanoma

With the growing understanding of genetic alterations in melanoma and immune modulation by T cell checkpoint molecules, two new therapeutic approaches have become standard therapy in late stage melanoma, both improving overall survival (OS) in randomized trials. Targeted therapy inhibiting the mutant BRAFV600 kinase by selective BRAF inhibitors vemurafenib or dabrafenib significantly improved PFS and OS as compared to “standard” chemotherapy DTIC in randomized phase 3 trials [14, 15]. Combined MAPK pathway targeting by BRAF (dabrafenib respectively vemurafenib) and MEK inhibition (trametinib respectively cobimetinib) improves OS further [16-18]. Therefore, treatment with dabrafenib + trametinib is currently being tested in the adjuvant setting (www.clinicaltrials.gov). However, targeted therapies rarely achieve long-term tumor control and tumor progression is observed

after median 6-9 months. Therefore it is questionable whether targeted therapy approaches will improve disease free survival in an adjuvant setting.

T cell checkpoint blockade, e.g. antibody-mediated blockade of CTLA-4 and PD-1/PD-L1, has become the most promising approach in treatment of late stage melanoma. Ipilimumab, a monoclonal antibody targeting CTLA-4, achieved improvement of OS in two randomized trials [10, 19]. While inducing tumor regression less frequently than targeted therapies, long-term benefit from immunotherapy is higher in the responding patients [20]. PD-1 blockade, by e.g. nivolumab or pembrolizumab, induces higher response rates than CTLA-4 blockade [21, 22]. Furthermore, the deepness of clinical responses (percentage of tumor load reduction) could be further enhanced by the combination of nivolumab and ipilimumab [7, 23], and response rate and progression-free survival was superior to ipilimumab or nivolumab monotherapy in a randomized phase 3 trial [9], and superior to ipilimumab in another randomized phase 2 trial [24].

Because of the profound clinical effects of checkpoint blockade and its ability to induce durable tumor control, immunotherapy seems to be the optimal treatment approach for adjuvant therapy in melanoma. Indeed, adjuvant ipilimumab significantly improved relapse-free and overall survival, and this e.g. to a higher absolute percentage than observed for herceptin in breast cancer [6, 25, 26]. Currently adjuvant trials comparing ipilimumab versus nivolumab and pembrolizumab versus placebo are implemented, but results are pending (www.clinicaltrials.gov). In addition, recently another trial has started testing neo-adjuvant and adjuvant nivolumab in stage III melanoma (NCT02519322).

Rationale for neo-adjuvant versus adjuvant immunotherapy

One major challenge, however, is the observation, that within the recently presented adjuvant trial testing ipilimumab, only 7% of patients completed the full scheme of ipilimumab treatment and maintenance for 3 years [27]. This indicates the need for more efficient and shorter adjuvant immunotherapy schemes, which could induce higher T cell immune responses against melanoma (neo-) antigens and subsequent higher disease-free survival rates.

In contrast to chemotherapeutic approaches or targeted therapy, immunotherapeutic approaches depend on sufficient activation of the immune system. To become fully activated, T cells require two signals [28]. The first signal is provided by the interaction of the (tumor-) antigen presented in the major histocompatibility complex (MHC) on the antigen-presenting cell (APC) with the T cell receptor (TCR) on the T cell (signal 1).

In parallel, a large number of co-inhibitory and co-stimulatory interactions – so-called T cell checkpoints - modulate the outcome of the TCR – pMHC interaction (signal 2) [29]. Antibody-

based interference with the T cell checkpoints CTLA-4 and PD-1 has been shown to improve tumor-specific T cell responses and to result in clinical benefit in patients with melanoma and other cancers [10, 21, 30]. T cell checkpoint inhibition (signal 2) is only of value at the moment of TCR triggering (signal 1) [29], which has potentially significant ramifications for the use of checkpoint targeting antibodies as adjuvant therapies. Specifically, as the amount of antigen that can provide this signal 1 will correlate with tumor load, we postulate that adjuvant immunotherapy will work most efficiently, when adjuvant treatment is initiated prior to surgery.

To directly address this question, the OpACIN trial has been set-up, in which we tested, in a phase 1 feasibility and safety study in resectable stage III melanoma patients, *neo-adjuvant plus adjuvant ipilimumab + nivolumab* short-term treatment (for sake of brevity also referred to as 'neo-adjuvant treatment') against short-term *adjuvant ipilimumab + nivolumab* treatment (Figure 2).

Figure 2

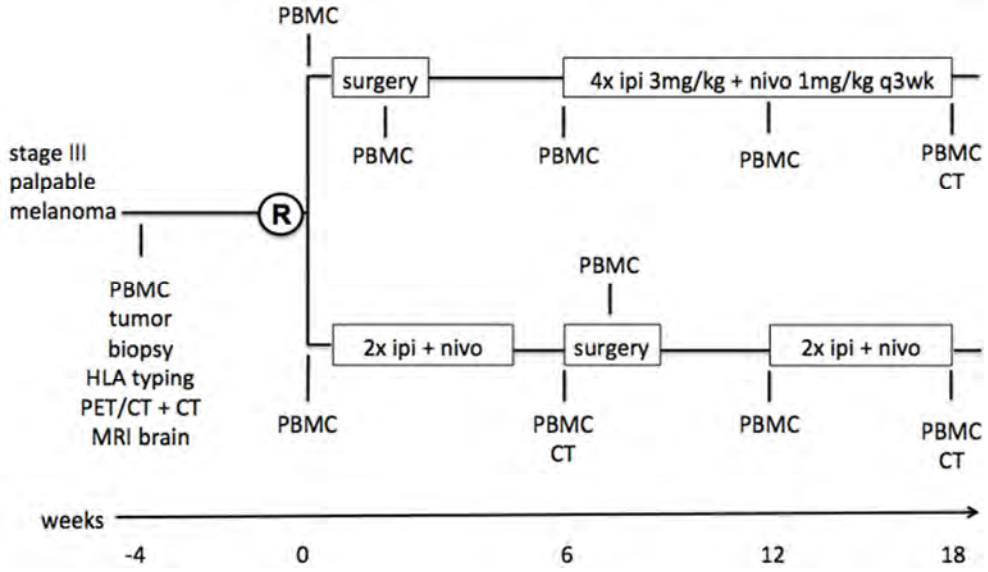

Figure 2. Schematic overview of the phase 1 OpACIN trial

This trial will finish recruitment in September 2016. Its primary objective was met, by showing, that it is feasible to treat patients neo-adjuvant with ipilimumab + nivolumab and perform the planned lymph-node dissection on time. However, in comparison to stage IV melanoma patients (where 55% of patients developed grade 3/4 toxicities, [9]), we observed grade 3/4 immune related adverse events (such as colitis, hypophysitis, hepatitis, dermatitis, and adrenal gland insufficiency) in more patients (so far 89% of the patients). For example,

one patient (that received 3 courses of ipilimumab + nivolumab) developed a severe Steven Johnson Syndrome, requiring long-lasting hospitalization (meanwhile recovered completely). Only 2/17 patients received so far all 4 courses of ipilimumab + nivolumab, due to onset of immune-related adverse events.

On the other side, 8/10 patients treated in the neo-adjuvant arm developed profound regressions of their macrometastases, including 3/10 pathological complete responses (pCR), and additional 3/10 near pCRs (remaining micrometastasis) and 2/10 pathological partial response (pPR) after the first 2 courses. This has led to the design of this phase 2 trial, maintaining the combination of ipilimumab and nivolumab (see rationale below), but now comparing different neo-adjuvant dosing schemes of only two courses ipilimumab + nivolumab for their safety and efficacy.

Rationale for Combining Nivolumab with Ipilimumab in Melanoma

Lymphocyte homeostasis is regulated by the differential effects of cytokines and an array of cell surface signaling molecules that affect T cell proliferation. One of the primary molecules responsible for T cell expansion in response to antigenic stimulation is CD28. The interaction of CD28 on the T cell, with CD80 (B7.1) or CD86 (B7.2) on the antigen presenting cell, stimulates T cell proliferation and survival (reviewed in [29]). By necessity, a mechanism for inhibition of antigen stimulated T cells is required following control of the antigenic challenge to maintain the viability of the host. One important regulator of T cell responses upon antigen stimulation is cytotoxic T lymphocyte antigen 4 (CTLA-4). CTLA-4 expression on T cells is increased following antigen stimulation and it has a significantly higher affinity for both B7 ligands than does CD28 [31]. CTLA-4 triggering leads to an inhibition of T cell proliferation and thereby helps to control T cell responses. The fact that CTLA-4 knockout mice display a lethal systemic immune hyper-activation demonstrates the important role of CLTA-4 in down-regulation of antigen-specific T cell responses [32].

Ipilimumab is a fully human monoclonal antibody that binds to CTLA-4 and inhibits its interaction with ligands on antigen presenting cells. Ipilimumab has been approved for use in over 40 countries including the United States in March 2011 and the European Union in July 2011. The safety profile is detailed in the Investigator Brochure. In brief, over 12,700 subjects received ipilimumab and the most common adverse events (AEs) were immune related in nature, consistent with ipilimumab mechanism of action, and were generally medically manageable with topical or systemic immunosuppressants.

Clinical trials with ipilimumab have shown activity in melanoma. In two phase 3 trials, ipilimumab was the first agent to demonstrate a statistically significant improvement in overall survival in subjects with metastatic melanoma [10, 19]. Monotherapy with ipilimumab at a

dose of 3 mg/kg improved overall survival compared to glycoprotein 100 peptide vaccine in subjects with previously treated metastatic melanoma (10.1 versus 6.4 months, hazard ratio 0.66, p-value 0.003) [10]. In the second study of subjects with treatment naive metastatic melanoma, overall survival was significantly longer in patients receiving dacarbazine with ipilimumab at a dose of 10 mg/kg, than in patients receiving dacarbazine with placebo (11.2 versus 9.1 months, hazard ratio 0.72, p-value < 0.001) [19].

A subsequent clinical trial testing the concurrent combination of nivolumab and ipilimumab resulted in even greater objective response rates than those seen with either agent alone in patients with advanced melanoma [8, 10, 33]. A total of 53 patients received concurrent therapy, resulting in an objective response rate of 40%. Evidence of clinical activity (conventional, unconfirmed, or immune related response or stable disease for ≥ 24 weeks) was observed in 65% of patients. At the maximum doses that were associated with an acceptable level of adverse events, 53% of patients had an objective response, all with tumor reduction of 80% or more. Treatment related adverse events were observed in 93% of subjects, with the most common events being rash (55%), pruritus (47%), fatigue (38%) and diarrhea (34%). Grade 3 or grade 4 treatment-related events were noted in 53% of subjects, with the most common events being elevated levels of lipase (13%), aspartate aminotransferase increased (13%) and alanine aminotransferase increased (11%). Six of 28 subjects (21%) had grade 3 or grade 4 treatment related events that were dose limiting. Eleven subjects (21%) discontinued therapy due to treatment related adverse events. The doses of nivolumab at 3 mg/kg and ipilimumab at 3 mg/kg exceeded the maximum tolerated dose due to the occurrence of asymptomatic grade 3 or grade 4 elevated levels of lipase that persisted for ≥ three weeks in three of six subjects. Doses of nivolumab at 1 mg/kg and ipilimumab at 3 mg/kg (n = 17 subjects), in addition to nivolumab at 3 mg/kg and ipilimumab at 1 mg/kg (n = 16 subjects) were both tolerated [23]. Additional clinical experience with over 250 subjects has shown that both combination regimens are tolerated in subjects with solid tumors, and the combination of ipilimumab 1 mg/kg + nivolumab 3 mg/kg seems to induce less frequent grade 3/4 toxicities (Table 1), although this has not yet been compared in a randomized manner. To address this question in late stage melanoma patients, the CA209-511 study has been set-up, which is currently recruiting (www.clinicaltrials.gov, NCT02714218).

In patients with advanced melanoma, concurrent treatment with the combination of nivolumab and ipilimumab results in rapid and deep regression of tumors in a substantial proportion of subjects, when compared to either agent alone. Even though 55% of subjects in the phase 3 study developed grade 3 or grade 4 adverse events upon ipilimumab 3 mg/kg + nivolumab 1 mg/kg, the safety profile was generally manageable, suggesting an acceptable

risk to benefit ratio [9]. Interestingly, the response rate was even higher (68%) in patients that developed grade 3/4 adverse events as compared to the whole population (58%) [9].

Table 1

Study	Grade 3 - 5 Drug-related Adverse Events	
	N1 + I3	N3 + I1
CA209016 Cohort 1	61%	29%
CA209016 Cohorts 1 & 2	64%	34%
CA209004 Cohort 2, 2a	77%	56%
CA209004 Cohort 8	66%	NA
CA209067	55%	NA
CA209069	54%	NA

Table 1: Summary of drug-related adverse events of combinations nivolumab 1 mg/kg + ipilimumab 3 mg/kg versus nivolumab 3 mg/kg + ipilimumab 1 mg/kg in different malignancies (source BMS, CA209-511 protocol)

Rationale for testing the three arms neo-adjuvant

The observation from OpACIN, that most of the patients did not receive 4 courses ipilimumab 3mg/kg + nivolumab 1mg/kg due to treatment–related toxicities, but also the observation that we observed a very high response rate after 2 courses in the neo-adjuvant arm, indicates, that stage 3 melanoma patients may benefit already from less infusions.

These observations have led to the design of this phase 2 trial that will test only 2 courses of neo-adjuvant ipilimumab + nivolumab with tighter discontinuation rules (see Chapters 6.7 - 6.9). We will test 3 different combination schemes with the aim to reduce toxicity while preserving efficacy.

The design of the additional combination schemes to be tested in this study (arm B and C) and compared to the standard combination schemes (arm A) are based on several observations from other early phase trials in stage 4 melanoma: a) in the phase 1 study testing ipilimumab + nivolumab in late stage melanoma also 4 courses of a lower dose of ipilimumab (1 mg/kg) were tested in combination with nivolumab 3 mg/kg indicating similar efficacy (CA209-004) [8], b) data from the Keynote 029 study testing low dose ipilimumab (1 mg/kg) with the standard dose of pembrolizumab (2 mg/kg) in 153 patients indicate that frequency of grade 3/4 toxicity is lower (to 42%), while disease control is preserved (G. Long, ASCO 2016 meeting), and c) data from a retrospective analysis of late stage melanoma

patients treated at the NKI with 2 courses ipilimumab 3 mg/kg q3wks, directly followed the next day by pembrolizumab or nivolumab at standard dosing (2 mg/kg q3wks or 3 mg/kg q2wks respectively). This sequential scheme also induced a similar disease control rate, as compared to the phase 3 trial testing the standard scheme ipilimumab + nivolumab [9], but was less toxic. We observed in only 38% of the patients grade 3/4 treatment-related toxicity (Meerveld, Rozeman, Blank, et al., submitted to Annals of Oncology). Subsequently, we regularly treated stage 4 melanoma patients with ipilimumab 3mg/kg for 1 or 2 courses, directly followed by nivolumab or pembrolizumab, starting on the same day after the ipilimumab infusion, observing no additional toxicities (manuscript in preparation).
Thus, the risk of neo-adjuvant immune related toxicity in OPACIN-neo is considered to be lower (mainly in arm B or C) than that of patients within the phase I OpACIN trial.

Rationale for radiological and pathological read-out as primary parameter

As described above, we have observed pathologic responses in 8/10 patients treated neo-adjuvant with ipilimumab 3 mg/kg + nivolumab 1 mg/kg . In 4/8 responding patients the CT response underestimated even the pathological response (Blank, et al. ESMO 2016). The high response rate, but also high rate of grade 3/4 toxicities, warrants further investigation of ipilimumab + nivolumab neo-adjuvant, but requires an analysis of alternative schemes (less toxic, but equally efficient). Therefore, the primary outcome measurements in this subsequent phase 2 trial will be toxicity, radiologic, and pathologic response rate in the three arms, and only in case of inconclusive results, we will analyze the broadening of the melanoma neo-antigen-specific T cell responses (as described below in background translational research and which will be performed in OpACIN).

Background translational research

Tumors such as melanoma and NSCLC that are responsive to T cell checkpoint blockade are characterized by large numbers of mutations, and such mutations can lead to the presentation of ‘neo-antigens’ that are foreign to the T cell-based immune system [34]. It was postulated already many years ago that T cell recognition of neo-antigens would form an important ingredient in T cell-based tumor recognition. However, the fact that the vast majority of the mutations within human tumors are patient-specific precluded the efficient analysis of neo-antigen specific T cell immunity until recently. Importantly, through the development of cancer exome sequencing and high throughput immunomonitoring technology (see below), it is now feasible to analyze neo-antigen specific T cell responses in individual patients and the effect of therapy on such responses. From these analyses we have learned that 1). CD8 T cell recognition (observed in 9 out of 11 patients analyzed; [35]

and van Buuren et al., unpublished) and CD4 T cell recognition (observed in 5 out of 6 patients) of neo-antigens is common in melanoma [36]. 2). neo-antigen specific T cell responses can be increased upon ipilimumab treatment [35], and 3). neo-antigen specific T cell responses can be induced or increased to detectable levels upon nivolumab treatment [37]. The ability to monitor the development/ enhancement of neo-antigen-specific T cell responses in patients undergoing treatment provides a unique opportunity to compare the effectiveness of different treatment strategies, in particular in situations in which a significant clinical readout is only available after long periods of time and with large numbers of patients. In case of similar radiological and pathological response rates of all three arms in this trial and in case of a similar toxicities observed, we will analyze patient material for intra-patient changes in neo-antigen specific T cell responses upon therapy.

To achieve the monitoring of neo-antigen specific T cell responses, the repertoire of mutations present within the melanoma of a patient is first analyzed by exome sequencing, using peripheral blood as reference material. In addition RNAseq is used to filter for expressed genes. With the list of patient-specific mutations in expressed genes in hand, potential neo-epitopes are predicted using an algorithm that takes into account predicted proteasomal processing, binding affinity for the HLA class I alleles carried by the patient, and 'dissimilarity to self'. This results in a list of potential neo-epitopes that is synthesized by classical peptide synthesis. Resultant peptides are loaded onto the relevant recombinant HLA class I alleles, using the ligand exchange technology developed by NKI [38]. Subsequently, these peptide HLA complexes are converted into multimers, labeled with fluorochromes and used for highly multiplexed T cell detection (combinatorial coding technology, also developed by NKI). This technology has successfully been used to identify neo-antigen specific T cell responses in patient material [35], and has a very high sensitivity/ reproducibility [39]. All identified T cell responses are routinely confirmed in an independent analysis. Analysis will be performed with the same setup/ filter steps as used in the OpACIN trial.

Another recent work from our collaborator Tom Wuerdinger has revealed the unprecedented potential of tumor educated platelets (TEPs) for pan-cancer blood-based diagnostics and molecular characterization Using the highly dynamic repertoire of TEPs, and the use of robust supervised machine learning algorithms, he identified TEP RNA signatures that predict with >95% accuracy whether an individuals has cancer or not (n=283 cancer patients, n=55 healthy donors), with 70% accuracy what tumor type is present (including 60 NSCLC patients, 41 colorectal cancer patients, 39 breast cancer patients, 39 glioblastoma patients, 35 pancreatic cancer patients, and 14 hepatobiliary cancer patients), and with high accuracy (80-90%) which molecular subtype (e.g. KRAS, EGFR, MET, HER2) is present in the

matching tumor tissue [40]. The RNA content of TEPs can be readily regarded as a dynamic mirror of tumor activity and a reflection of the responses in the bodies' environment. Thus TEP could become another biomarker for outcome upon melanoma immunotherapy, and might outperform RNA-signatures from tumor biopsies. Thus during this trial, EDTA blood will be collected for TEP analysis and circulating DNA analysis.

Risk benefit discussion

Currently there is no standard adjuvant therapy for stage III melanoma. Post-surgery adjuvant radiotherapy is commonly applied, because it has been shown to marginally improve local disease control, but no improvement in relapse-free survival or overall survival was observed in this high-risk population [1, 2]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable survival benefit (two out of three meta-analyses found no OS benefit [3-5]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe. Adjuvant systemic immunotherapy in form of ipilimumab has been tested with promising results in stage III melanoma [6], however overall survival benefit has not been shown so far, and toxicity was high, mainly when applied longer than four courses.

Participants of this study will be exposed to two immunotherapeutic agents (ipilimumab, nivolumab) known to induce immune related adverse events at a high percentage (more than 50%) when combined together [9]. In this phase 3, trial 99.7% of the 313 patients experienced adverse events (of any grade). 95.5% of adverse events were reported as treatment related, the most common ones being diarrhea (44.1%), rash (40.3%), fatigue (35.1%), and pruritus (33.2%). Treatment related grade 3 and 4 adverse events were observed in 55%, including hepatic, gastro-intestinal, renal toxicity and more rarely pneumonitis and uveitis.

In analogy to the ipilimumab adjuvant trial [6, 10], we showed that (neo)-adjuvant administration of ipilimumab + nivolumab to patients with stage III melanoma leads to a higher frequency of adverse events when compared to patients with stage IV disease. Specifically, within the precursor phase 1 trial (OpACIN, CA209-278), we observed that only 2/18 patients received all planned 4 courses of ipilimumab + nivolumab due to immune-related toxicities. In detail 16/18 (89%) so far evaluable patients (9 neo-adjuvant arm, 7 adjuvant arm) developed one or more immune related adverse events grade 3 or higher (7x elevated lipase, 6x colitis, 4x hepatitis, 3x endocrinopathies, 2x dermatitis 1x delirium due to immune related meningitis, 1x Steven Johnson Syndrome, , 1x hyperglycemia), that all have

recovered to grade 1 or fully recovered from their symptoms, but require ongoing hormonal substitution.

While such immune-related adverse event could hamper on-time surgery, we have not observed this within our OpACIN phase 1 trial, as all neo-adjuvant patients have undergone lymph node dissection on time at week 6.

In addition, without local radiotherapy the patients treated within this trial with systemic immunotherapy bear the risk of reduced local tumor control. On the other side, the patients have the chance of improved RFS and OS, based on a) the observation that single ipilimumab improved RFS and OS in a phase 3 trial, b) that the combination of ipilimumab + nivolumab induces superior response rates as compared to ipilimumab alone in stage 4 melanoma [8, 10, 27], and c) on the observed high response rate in patients treated neo-adjuvant in the phase 1 OpACIN trial.

Finally, post-surgery recovery could theoretically be altered when applying neo-adjuvant ipilimumab + nivolumab. This was not observed in our phase 1 trial, and was also not observed in an another analysis of patients undergoing surgery during ipilimumab treatment [41].

On the benefit side, of the 10 patients treated in the neo-adjuvant arm of OpACIN, 5/10 patients had a PR, 2/10 SD, and 1/10 PD on CT-scan according to RECIST 1.1after the first 2 courses (in 2 patients there was no CT-scan performed before surgery). Moreover, 8/10 patients developed profound pathological regression of their macrometastasis, 3/10 pCRs, 3/10 near pCR (remaining micrometastasis), and 2/10 pPR (remaining metastasis of 0.5 and 9 mm).

This has led to the current design of this phase 2 trial, maintaining the combination of ipilimumab and nivolumab, but now comparing three neo-adjuvant schemes containing only 2 courses ipilimumab + nivolumab with the aim of maintaining this high efficacy, while reducing the frequency of grade 3/4 toxicities.

2. OBJECTIVES

Primary objectives:

- To compare safety (immune related adverse events during the first 12 weeks) of three different neo-adjuvant combination schemes of ipilimumab + nivolumab
- To compare radiological and pathological response rates at week 6 of the different neo-adjuvant combination schemes

Primary endpoints:

- Safety as measured by the frequency of grade 3/4 immune-related adverse events (during the first 12 weeks).
- Response rate according to RECIST 1.1 at week 6
- Pathological response according to central pathological revision (complete response, near complete response [only single tumor cells remaining], partial pathological response /micrometastasis, macroscopic disease)

An interim analysis will be performed after 13 patients have been included in each arm (see 4.5), thus in total 39 patients have been included.

Secondary objectives:

- To describe relapse free survival
- To describe rate and type of late adverse events
- To determine immune activating capacity of the alternative combination schemes

Secondary endpoints:

- Recurrence Free Survival.
- Description of late adverse event (irAE, up to 3 years after treatment initiation, until new treatment) according to CTCAE v4.03
- Description of associations of mutational load, RNA tumor signatures, and tumor educated platelet signatures with tumor immune infiltrates and response
- Alteration in magnitude or breadth of the neo-antigen specific T cell responses in peripheral blood from baseline to surgery at week 6 in each 10 randomly chosen patients per arm.

3. STUDY DESIGN

This is an open-label three-arm phase 2 trial (including a Simon stage 2 design, see also 4.4 and 4.5) consisting of 90 stage III melanoma patients randomized 1:1:1 to receive 2 courses standard combination of ipilimumab 3 mg/kg + nivolumab 1 mg/kg q3wk (arm A), 2 courses ipilimumab 1 mg/kg + nivolumab 3 mg/kg q3wk (arm B), or 2 courses of ipilimumab 3 mg/kg q3wks, directly followed (> 2 hours and < 24 hours) by 2 courses nivolumab 3 mg/kg every 2 weeks (arm C) prior to surgery at week 6 (30 patients per arm, Figure 3). Patients will be stratified according to treatment center.

After 39 patients (13 per arm) the Data Safety Monitoring Board (DSMB) will be informed about the observed efficacy and toxicity within each arm and give advice, based on this information, whether the study will be continued (see also 4.5).

Figure 3

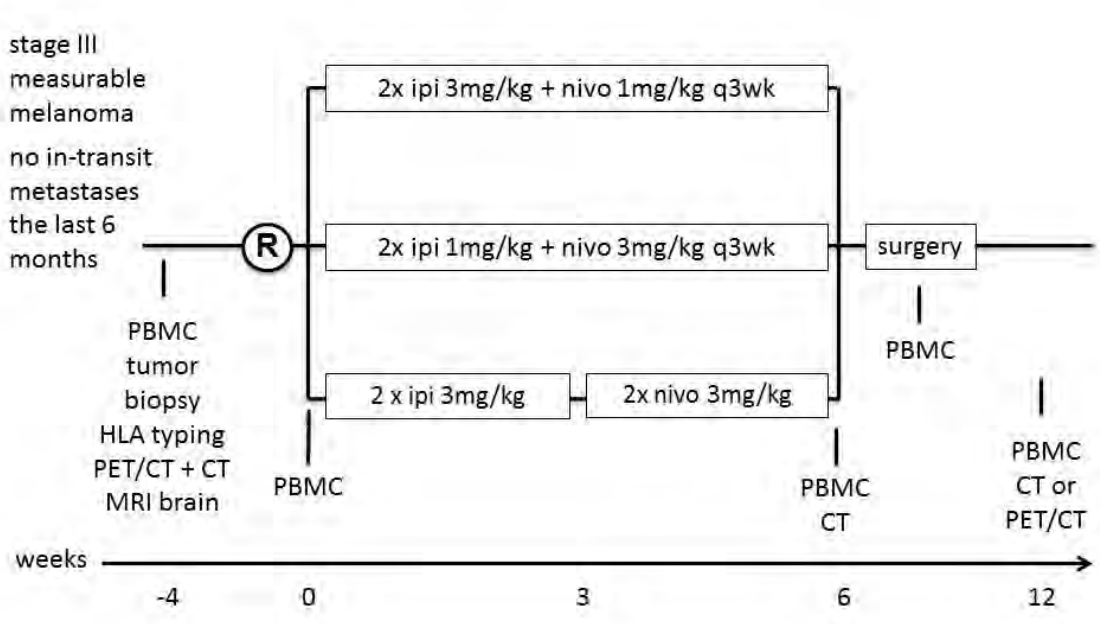

Figure 3: Schematic overview of the phase 2 OpACIN-neo study

4. STUDY POPULATION

4.1 Population

Resectable stage III melanoma patients with one or more measurable lymph node metastases (according to RECIST 1.1) that can be biopsied, no history of in-transit metastases within the last 6 months, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, and more than 18 years old.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Adults at least 18 years of age
- World Health Organization (WHO) Performance Status 0 or 1
- Cytologically or histologically confirmed resectable stage III melanoma with one or more macroscopic lymph node metastases (measurable according to RECIST 1.1), that can be biopsied, and no history of in-transit metastases within the last 6 months
- No other malignancies, except adequately treated and a cancer-related life-expectancy of more than 5 years
- Patient willing to undergo triple tumor biopsies and extra blood withdrawal during screening and in case of relapse
- No prior immunotherapy targeting CTLA-4, PD-1 or PD-L1
- No immunosuppressive medications within 6 months prior study inclusion
- Screening laboratory values must meet the following criteria: WBC ≥ $2.0x10^9$/L, Neutrophils ≥$1.5x10^9$/L, Platelets ≥100 $x10^9$/L, Hemoglobin ≥5.5 mmol/L, Creatinine ≤1.5x ULN, AST ≤ 1.5 x ULN, ALT ≤ 1.5 x ULN, Bilirubin ≤1.5 X ULN
- Normal LDH
- Women of childbearing potential (WOCBP) must use appropriate method(s) of contraception. WOCBP should use an adequate method to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug
- Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of ipilimumab + nivolumab

- Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product
- Women who are not of childbearing potential (i.e., who are postmenopausal), or surgically sterile as well as azoospermic men do not require contraception
- Patient is capable of understanding and complying with the protocol requirements and has signed the Informed Consent document.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Distantly metastasized melanoma
- History of in-transit metastases within the last 6 months
- No measurable lesion according to RECIST 1.1
- Subjects with any active autoimmune disease or a documented history of autoimmune disease, or history of syndrome that required systemic steroids or immunosuppressive medications, except for subjects with vitiligo or resolved childhood asthma/atopy
- Prior CTLA-4 or PD-1/PD-L1 targeting immunotherapy
- Radiotherapy prior or post-surgery
- Patients will be excluded if they test positive for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody), indicating acute or chronic infection
- Patients will be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS)
- Allergies and Adverse Drug Reaction
  - History of allergy to study drug components
  - History of severe hypersensitivity reaction to any monoclonal antibody
- Underlying medical conditions that, in the Investigator's opinion, will make the administration of study drug hazardous or obscure the interpretation of toxicity determination or adverse events;
- Concurrent medical condition requiring the use of immunosuppressive medications, or immunosuppressive doses of systemic or absorbable topical corticosteroids;
- Use of other investigational drugs before study drug administration 30 days and 5 half-times before study inclusion

• Pregnant or nursing

4.4 Sample size calculation

The primary objectives of the study are to compare the frequencies of treatment-related grade 3/4 toxicities and compare efficacy (response rate) of three different neo-adjuvant schemes of ipilimumab plus nivolumab: 2 courses standard combination of ipilimumab 3 mg/kg + nivolumab 1 mg/kg q3wk (arm A), 2 courses ipilimumab 1 mg/kg + nivolumab 3 mg/kg q3wk (arm B), or 2 courses of ipilimumab 3 mg/kg q3wks, directly followed (> 2 hours and < 24 hours) by 2 courses nivolumab 3 mg/kg every 2 weeks (arm C) prior to surgery at week 6 (30 patients per arm).

Based on data from our previous study (OpACIN) we assume a true grade 3/4 immune-related toxicity rate of 89%, and any grade toxicity rate of 100%, and a pathological response rate of 80% for patients in Arm A.

Assuming 89% grade 3/4 toxicity of the current schedule, a Fisher's exact test with two groups of 30 patients each will have 89% power at a 0.05 two-sided significance level to detect a difference in toxicity to a proportion grade 3/4 toxicity of 50% or lower.

Assuming a pathological response rate of 80% with the current schedule, the lower 95% confidence interval of the response rate in a group of 30 patients will extend to 61% (based on an exact binomial test). A response rate lower than that suggests that the alternative schedule is less effective. In this setting, arm B will be compared to arm A and arm C will be compared to arm A.

4.5 Interim analysis

Although an improvement in safety (lower grade 3-4 toxicity), the primary endpoint of the study, is expected in arm B and C compared to arm A, it should at the same time, not have a detrimental effect on response rate. In arm A, a response rate of 80% is expected. An interim analysis will be planned using a Simon 2-stage approach within arm B and separately within arm C with the focus on response rate. It is suggested to present these data to the DSMB (together with safety data). The following rule will be applied: The pathological response rate that clearly implies that the treatment does not warrant further investigation is set to 50%. This will be tested against a one-sided alternative of 75% (which is considered sufficiently interesting). In
the first stage, 13 patients per arm, thus in total 39 patients will be accrued. If there are 6 or fewer responses within the 13 patients per arm, then this arm will be closed. In that case, it has to be concluded that the chance that the response is worse than 50% is too high

(significance level 0.025). Otherwise, 17 additional patients will be accrued for a total of 30 patients. The response rate of 50% (or lower) will be rejected in favor of the alternative response rate if 21 or more responses are observed in 30 patients with a power of 80%.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Patients will be treated with 2 courses ipilimumab 3 mg/kg and nivolumab 1 mg/kg, every 3 weeks (Arm A), 2 courses ipilimumab 1 mg/kg and nivolumab 3 mg/kg, every 3 weeks (Arm B), or 2 courses of ipilimumab 3 mg/kg, every 3 weeks directly followed (> 2 hours interval, but within 24 hours) by nivolumab 3 mg/kg every 2 weeks (Arm C)

5.2 Use of co-intervention

Prohibited medication:

1) Concurrent chemotherapy, hormonal therapy (oral anti-conception is permitted), immunotherapy regimens, or radiation therapy, standard or investigational.

2) Use of growth factors including, but not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), or erythropoietin stimulating agents is not permitted, unless deemed necessary by investigator and discussed with medical monitor.

3) Use of systemic corticosteroids at > 10 mg daily prednisone equivalent, unless required for the treatment of infusion reactions, other adverse events, or for palliation as determined by the investigator.

4) Steroids must not be given as prophylactic anti-emetic therapy.

5) Use of herbal remedies is not permitted

Permitted medication:

The use of prescription and over-the-counter medications (except medications from categories outlined above) is permitted at the discretion of the investigator and must be recorded on CRF.

Subjects are permitted to use topical, ocular, intranasal, intra-articular, and inhalational corticosteroids (with minimal systemic absorption). Immunosuppressive doses (e.g., prednisone > 10 mg/day or equivalent) and/or physiologic replacement doses of systemic corticosteroids (e.g., prednisone <10 mg/day) are permitted in the context of treating adverse events. A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

Prophylactic anti-emetics, with the exception of steroids, may be administered at the discretion of the treating physician before any doses of study drug.

Use of the seasonal killed influenza vaccine during therapy is permitted without restriction. However, influenza vaccines containing live attenuated virus (Flumist ®) or other clinically indicated vaccinations for infectious diseases (killed or attenuated, e.g., Pneumovax ®, varicella, MMR, etc) may be permitted, but must be discussed with the PI and may require a study drug washout period prior to and after administration of the vaccine.

Contraception:

A Woman of Childbearing Potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, females under the age of 55 must have a serum follicle stimulating hormone, (FSH) level > 40mIU/mL to confirm menopause.

Females treated with hormone replacement therapy, (HRT) are likely to have artificially

suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is > 40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products, (rings, creams, gels)
- 4 week minimum for transdermal products
- 8 week minimum for oral products

Other parenteral products may require as long as 6 months.

5.3 Escape medication (if applicable)

All immune related adverse events should be treated according to standard algorithms (see appendix).

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Nivolumab 3 or 1 mg/kg, every 2 or 3 weeks, administered 2 times (6 weeks in total).

Ipilimumab 1 or 3 mg/kg, every 3 weeks, administered 2 times (6 weeks in total).

Treatment schedule			
week	Arm A	Arm B	Arm C
-4 till 0	Screening/Baseline	Screening/Baseline	Screening/Baseline
0	nivolumab 1 mg/kg ipilimumab 3 mg/kg	nivolumab 3 mg/kg ipilimumab 1 mg/kg	ipilimumab 3 mg/kg
1	x	x	x
2	x	x	x
3	nivolumab 1 mg/kg ipilimumab 3 mg/kg	nivolumab 3 mg/kg ipilimumab 1 mg/kg	ipilimumab 3mg/kg followed by (>2h and <24h) nivolumab 3 mg/kg
4	x	x	x
5	x	x	nivolumab 3 mg/kg
6	**surgery**	**surgery**	**surgery**

Product Description and Dosage Form	**Potency**	**Primary Packaging (Volume)/ Label Type**	**Secondary Packaging (Qty) /Label Type**	**Appearance**	**Storage Conditions (per label)**
Nivolumab/BMS-936558-01 Solution for Injection	100 mg (10 mg/mL)	10 mL/open label	10 vials per box/ open labels	Colorless to pale yellow, clear to opalescent solution; may contain particles	Store Refrigerated, 2-8° C (36-46 F) Protect from light and freezing.
Ipilimumab Solution for Injection	200 mg (5 mg/mL)	40 mL per vial/Open label	4 vials per carton/Open label	Clear, colorless to pale yellow liquid. May contain particles	2 to 8°C. Protect from light and freezing.

6.2 Preclinical Summary of Nivolumab combined with Ipilimumab

Preclinical data indicate that the combination of PD-1 and CTLA-4 receptor blockade may improve antitumor activity. In vitro combinations of nivolumab plus ipilimumab increase IFN- production 2- to 7-fold over either agent alone in a mixed lymphocyte reaction. Increased antitumor activity of the combination was also observed in 3 of 5 syngeneic murine cancer models. In a murine melanoma vaccine model, blockade with either CTLA-4 or PD-1 antibodies increased the proportion of CTLA-4 and PD-1-expressing CD4/CD8 tumor infiltrating T effector cells, and dual blockade increased tumor infiltration of T effector cells and decreased intratumoral T regulatory cells, as compared to either agent alone [42].

Preclinically, a 4-week toxicity study of nivolumab in combination with ipilimumab conducted in cynomolgus monkeys demonstrated that the combination of nivolumab and ipilimumab resulted in dose-dependent gastrointestinal (GI) toxicity. Histologic findings included inflammatory changes in the large intestine, which increased in incidence and severity in a dose-dependent manner. GI toxicity/colitis was not observed in cynomolgus monkeys administered nivolumab alone, but was observed in monkeys receiving ipilimumab monotherapy. Nivolumab in combination with ipilimumab was also associated with lymphoid hypocellularity of the cortex and/or medulla of the thymus and with acinar cell degranulation in the pancreas. Additional findings included interstitial mononuclear cell infiltrates in the kidneys, portal mononuclear cell infiltrates in the liver and myeloid hypercellularity in the bone marrow. Nivolumab in combination with ipilimumab at the high-dose level (ie, 50 mg/kg and 10 mg/kg, respectively) was associated with the death of 1 animal, attributed to acute gastric dilatation without histopathological evidence of colitis upon pathology evaluation of the GI tract.

6.3 Summary of findings from clinical studies

Ipilimumab Monotherapy

In MDX010-20, the ipilimumab monotherapy arm was administered 3 mg/kg ipilimumab every 3 weeks for four doses. In this arm, there were 79% drug related adverse events, with 21% being Grade 3/4 and 3/131 (2%) Grade 5. The most frequent adverse events of interest were rash (30%), pruritus (33%), diarrhea (33%), colitis (8%), endocrine disorders (9%), AST/ALT increased (2%), and hepatitis (1%). Any grade immune related adverse events were 60% and the Grade 3/4 immune related adverse events for the same cohort was 13% with the most frequent adverse events being diarrhea (5%), colitis (5%), rash (2%), and endocrine disorders (3%). Additional details on the safety profile of ipilimumab, including results from other clinical studies, are also available in the ipilimumab IB.

Nivolumab Monotherapy

One study has contributed most to the clinical experience with nivolumab monotherapy in subjects with melanoma and other solid malignancies. CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 304 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 03-Jul-2012, a total of 107 melanoma subjects were treated with nivolumab in the dose range of 0.1-10 mg/kg. No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 72.4% of subjects. The most frequent nivolumab related AEs occurring in ≥5% of subjects included: fatigue (25.7%), rash (13.5%), diarrhea (11.8%), pruritis (10.2%), nausea (7.9%), decreased appetite (7.9%), hemoglobin decreased (5.9%) and pyrexia (5.3%). The majority of events were low grade, with grade 3-4 drug related AEs observed in 14.8% of subjects. The most common Grade 3-4 drug-related AEs occurring in ≥ 1% of subjects were: fatigue (1.6%), lymphopenia (1.3%), abdominal pain (1%), diarrhea (1%), hypophosphatemia (1%) and pneumonitis (1%). At least one SAE was reported for 150 (49.3%) of the 304 subjects at all dose levels. Grade 3-4 SAEs were reported for 23 subjects (7.6%). Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%). Additional select treatment-related AEs have occurred with low frequency (< 5%) but are considered clinically meaningful, as they require greater vigilance for early recognition and prompt intervention. These AEs include: ALT increased (4.3%), AST increased (3.6%), pneumonitis (3.3%), hypothyroidism (3.0%), hyperthyroidism (1.3%), renal failure (1.0%), adrenal insufficiency (0.7%) and colitis (0.7%). Grade 3-4 events of pneumonitis were reported in 3 subjects (1.0%) as described above (1 event was Grade 4). Grade 3 events of colitis, ALT increased, and AST increased were reported in 2 subjects (0.7%) each. Grade 3 events of adrenal insufficiency, hyperthyroidism, and hypothyroidism were reported in 1 subject (0.3%) each. Treatment-related AEs leading to discontinuation were reported in 18 (5.9%) of the 304 treated subjects on CA209003. The only events reported in more than 1 subject were pneumonitis (4 subjects; 1.3%) and hepatitis (2 subjects; 0.7%). There were 3 (1%) drug related deaths; each occurred after development of pneumonitis. Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported.

The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in nivolumab exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with nivolumab during pregnancy. Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the nivolumab IB.

Nivolumab Combined with Ipilimumab.

In the Phase 1 study CA209004, ascending doses of nivolumab have been studied concomitantly with ascending doses of ipilimumab in subjects with unresectable or metastatic melanoma. In each arm in this multi-arm study, ipilimumab was administered once every 3 weeks for 4 doses with nivolumab administered once every 3 weeks for 8 doses. Starting at week 24, ipilimumab and nivolumab were administered once every 12 weeks for 8 doses. The three initial dose-escalation cohorts consisted of Cohort 1 (nivolumab 0.3 mg/kg plus ipilimumab 3 mg/kg; n=14), Cohort 2 (nivolumab 1.0 mg/kg plus ipilimumab 3 mg/kg; n=17) and Cohort 3 (nivolumab 3.0 mg/kg plus ipilimumab 3 mg/kg; n=6). Later, the study was amended to include Cohort 2a which evaluated nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (n-16). The following DLTs were observed in Cohort 1 - Grade 3 elevated AST/ALT (1 subject); in Cohort 2 - Grade 3 uveitis (1 subject) and Grade 3 elevated AST/ALT (1 subject) and in Cohort 3 - Grade 4 elevated lipase (2 subjects) and Grade 3 elevated lipase (1 subject). Based on these data, Cohort 2 was identified as the maximum tolerated dose (MTD) and Cohort 3 exceeded the MTD. As of 15-Feb-2013, a total of 53 melanoma subjects were treated with nivolumab combined with ipilimumab in CA209004 across cohorts 1, 2, 2a, and 3. At least one AE regardless of causality has been reported in 98% of subjects treated. The most common (reported at > 10% incidence) treatment related AEs (any Grade %; Grade 3-4 %: 93; 53) are rash (55; 4), pruritus (47; 0), vitiligo (11; 0), fatigue (38; 0), pyrexia (21, 0), diarrhea (34; 6), nausea (21, 0), vomiting (11, 2), ALT increased (21; 11), AST increased (21; 13), lipase increased (19; 13), amylase increased (15, 6), headache (11, 0), and cough (13, 0).

The majority of AEs leading to discontinuation (regardless of causality) were Grade 3 or 4 (reported in 11 of 53 subjects, 21%). Grade 3 events included lipase increased, ALT increased, AST increased, troponin I increased, colitis, diverticular perforation, pancreatitis, tachycardia, renal failure acute, choroiditis, autoimmune disorder, and pneumonitis. One subject each discontinued due to Grade 4 events of blood creatinine increased and AST increased. No drug-related deaths were reported.

6.4 Summary of known and potential risks and benefits

There is no standard systemic adjuvant immunotherapy for stage III melanoma. Participation in this trial sets the patients at high risk of developing immune related adverse events. Algorithms have been developed to treat patients developing irAEs. Recovery is commonly observed (except for endocrine irAE) and depends on the fast onset of the advised immunosuppression.

Local recurrences could be at a slightly higher rate due to neglecting local radiotherapy.

Systemic recurrences and overall survival could be improved by treatment with ipilimumab+nivolumab based on the promising data in stage IV melanoma patients [9], RFS and OS benefit data from the phase 3 study of adjuvant ipilimumab after surgical treatment for stage III disease [6], and the high response rates observed in the phase 1 trial testing ipilimumab + nivolumab neo-adjuvant (OpACIN).

6.5 Description and justification of route of administration and dosage

Both ipilimumab and nivolumab are monoclonal antibodies and need to be i.v. infused, at dosing as described in 6.6.

6.6 Dosages, dosage modifications and method of administration

In combination with ipilimumab, nivolumab is to be administered always first (arm A and B). In arm C nivolumab should be infused within 24h after ipilimumab with an observation time of at least 2h in between.

Subjects should receive nivolumab at a dose of 1 mg/kg (Arm A) or 3 mg/kg (Arm B, C) as a 30-minute IV infusion.

The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the next infusion.

The second infusion when given in combination will always be ipilimumab, and will start at least 30 minutes after completion of the nivolumab infusion. Subjects should receive ipilimumab at a dose of 3 mg/kg (Arm A, C) or at a dose of 1 mg/kg (Arm B) as a 30-minute IV infusion.

The risk/benefit profile for nivolumab has primarily been investigated using a 60-minute infusion and for ipilimumab a 90-minute infusion. Long infusion times place a burden on patients and treatment centers. Establishing that these agents can be safely administered using shorter infusion times will diminish some of this burden.

Previous clinical studies of nivolumab have used 60-minute infusion duration and for ipilimumab 90-minute. Both nivolumab and ipilimumab have been administered safely at doses ranging up to 10 mg/kg over these treatment durations. Overall, infusion reactions including high-grade hypersensitivity reactions have been uncommon across multiple clinical studies, and all have been managed by following the safety algorithms. Infusion duration of 30 minutes for 3 mg/kg nivolumab (30% of the dose provided at 10 mg/kg) is not expected to present any safety concerns compared with the prior experience at 10 mg/kg nivolumab dose infused over the 60-minute duration.

Similarly, shortened infusion duration of 30 minutes for ipilimumab is not expected to present additional safety concerns, as described recently [43].

6.7 Dose Delay Criteria

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories (see appendices).

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab, ipilimumab or both). All study drugs must be delayed until treatment can resume.

Dose delay may not interfere with the timing of surgery. In that case the remaining dose will not be applied. There is no post-surgery application allowed.

Nivolumab and ipilimumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase, AST, ALT, or total bilirubin:
    - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay. It is recommended to consult with the principle investigator for Grade 3 amylase or lipase abnormalities.

- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

6.8 Criteria to Resume Treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- **If resumed infusion date would be less than 1 week before planned surgery, patients may not resume treatment**
- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (Section 6.9) should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis (<grade 3) must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

6.9 Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy
- **Grade 2 colitis lasting > 7 days or requiring any steroid treatment (inclusive local application)**

- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- **Any dosing interruption lasting > 2 weeks**
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab or ipilimumab dosing

6.10 Treatment of Nivolumab or Ipilimumab Related Infusion Reactions

Since nivolumab and ipilimumab contain only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE 4.03 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

Stop the nivolumab or ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab or ipilimumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab or ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 100 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of nivolumab or ipilimumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1

mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab or ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.11 Preparation and labeling of Investigational Medicinal Product

Preparation and labeling of the investigational medicinal products will be performed by the pharmacy of the participating center according to in-house SOPs.

6.12 Drug accountability

Drug accountability will be performed by the pharmacy of the participating centers according to internal standards.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Primary readout will be safety as measured by immune-related grade 3/4 adverse events (during the first 12 weeks).

Additional primary read-outs will be response rate according to RECIST 1.1 at week 6 and pathological response (according to centrally revised pathological examination).

An interim analysis will be performed after 13 patients have been included in each arm (see 4.5), thus in total 39 patients have been included.

7.1.2 Secondary study parameters/endpoints

Recurrence Free Survival

Rate and type of late adverse events (irAE, up to 3 years after treatment initiation until new treatment)

Description of associations of mutational load, tumor RNA, and tumor educated platelet signatures with tumor immune infiltrates and response.

Alteration in magnitude or breadth of the neo-antigen specific T cell responses in peripheral blood from baseline to surgery at week 6 in 10 patients treated in each arm (optional).

7.1.3 Other study parameters (if applicable)

n. a.

7.2 Randomization, blinding and treatment allocation

Patients fulfilling the eligibility criteria will be enrolled in the study. Patients will be randomized to receive either 2 courses ipilimumab 3 mg/kg + nivolumab 1 mg/kg q3wk (arm A), 2 courses ipilimumab 1 mg/kg + nivolumab 3 mg/kg q3wk (arm B), or 2 courses of ipilimumab 3 mg/kg every 3 weeks, directly followed (>2 hours and < 24 hours) by 2 courses nivolumab 3 mg/kg every 2 weeks (arm C). Patients will be stratified according to treatment center.

There is no blinding.

7.3 Study procedures

An overview of the study procedures can also be found in Table 2.

7.3.1 Screening phase

Following signing of the informed consent form for screening and enrolment into the study, the remainder of screening procedures and tests will be completed.

- Complete physical examination including height, weight, performance status, and vital signs, noting in detail the exact size and location of any lesions that exist.
- Medical history
- Tumor biopsies (3x 14g: 2x frozen, 1x FFPE) for pathological confirmation of stage 3 melanoma and genetic analysis of tumor. Patients may enter the study with a pathologic diagnosis of melanoma from any institution. Diagnosis must then be confirmed on the new biopsies by a pathologist of the participating center. If the diagnosis is not confirmed, the patient will be excluded from the study and replaced by another patient.
- Hematology (Hb, platelet count, absolute neutrophil count (ANC), white blood cell diff, Hct), PT/INR aPTT, Chemistry (LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, glucose, amylase, lipase, TSH, fT4, ACTH, cortisol, LH, FSH, S100, CRP, ESR)
- Baseline PET/CT and brain MRI as standard of care; an additional diagnostic CT of the neck, chest, abdomen and pelvis is required
- ECG
- HIV antibody titer, HbsAg determination, Anti-HCV, anti-CMV antibody titer
- beta-HCG pregnancy test
- PBMC's (see Appendix for CIMT protocol, deviations according to own centers' standard protocol are allowed), EDTA blood (for isolation of plasma and thrombocytes)will be taken twice before start of treatment (once during screening and once 0-2 days before start of treatment)

7.3.2 During treatment

- The patients will be treated pre-surgery to receive either 2 courses ipilimumab 3 mg/kg + nivolumab 1 mg/kg every 3 weeks (Arm A), 2 courses ipilimumab 1 mg/kg + nivolumab 3 mg/kg every 3 weeks (Arm B), or 2 courses ipilimumab 3 mg/kg, 3 weeks directly followed (> 2 hours and , 24 hours) by 2 courses nivolumab 3 mg/kg (Arm C) (see also Chapter 6.1).
- Targeted physical examination including weight, performance status, and vital signs week 0, 3, 6, 9,12
- Hematology (Hb, white blood cell diff, platelet count, Hct), Chemistry (LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, glucose, lipase, amylase, TSH, fT4, ACTH, cortisol, LH, FSH, S100, CRP, ESR)

  week 0, 3, 6, 9, 12
- beta-HCG pregnancy test before every cycle
- diagnostic CT of the neck, chest, abdomen and pelvis week 6
- From all patients PBMC's (see Appendix for CIMT protocol, deviations according to own centers' standard protocol are allowed), and EDTA blood (for isolation of plasma and thrombocytes) will be taken week 6 pre-surgery and during surgery (after tumor removal), and week 12.
- At week 6 patients will undergo lymph node dissection (and primary melanoma resection, if not resected previously). **If possible surgical material should be preserved as frozen and FFPE material.**
- Adverse events

7.3.3 Surgery at week 6

- The resection of Stage III lymph nodes must be performed in complete compliance with the Criteria for adequate surgical procedures for therapeutic lymph node dissection (TLND).
- To be considered as adequate, the surgical and pathological procedures should include at least the following:

Head and Neck

- Minimum of 15 pathologically investigated nodes
- Face, ear, and anterior scalp: parotidectomy plus (modified) radical neck dissection
- Posterior scalp: (modified or posterior) radical neck dissection plus suboccipital nodes. For this specific localization, a TLND will be considered as adequate if at least 5 LN have been investigated

Upper Extremity

- Minimum of 10 pathologically investigated nodes
- Axillary node dissection included at least 10 nodes taken from Levels I and II
- It is advised to routinely include the Level III nodes too
- Level III nodes should always be dissected if they were clinically involved

Lower Extremity

- Minimum of 5 pathologically investigative nodes
- If Cloquet's node is positive, a deep groin (external iliac and obturator) node dissection should be performed

(Modified) Radical Neck Dissection:

Classic or modified radical neck dissection may be performed for patients with melanoma of the head and neck. Minimum of 15 nodes must be pathologically investigated. As noted above, patients with melanoma located on the ear and anterior scalp and face will require superficial parotidectomy along with a (modified) radical neck procedure. The boundaries of the radical neck dissection are inferiorly the clavicle; the mandible, the mastoid and the tail of the parotid gland superiorly; the anterior border of the trapezius muscle posteriorly and the strap muscle of the larynx anteriorly. The sternocleidomastoid muscle, internal jugular vein and/or spinal accessory nerve may be sacrificed or preserved at the surgeon's discretion. For

posterior lesions the radical neck incision must be extended posteriorly or a second incision must be made so that the sub-occipital / postauricular nodal group can be sampled. For posterior neck dissection, surgical and pathological exploration of 5 nodes are sufficient to consider the procedure is adequate.

Axillary Lymphadenectomy:

For this study a complete axillary lymph node dissection will be performed including nodes at levels I, II and III (level III does not have to be removed routinely, but is advised. In any case level III should be dissected in case of preoperative nodal involvement). Axillary node dissection must include at least 10 nodes taken from Levels I and II and the level III nodes dissected for palpable axillary nodes. Minimum of 10 nodes must be pathologically investigated. The boundaries of the dissection should include the axillary vein superiorly beginning at the thoracic outlet and coursing to the latissimus dorsi tendon. The lateral border of the dissection is the anterior edge of the lattisimus dorsi muscle. The posterior boundary is the subscapular muscle. The anterior border of the resection is the pectoralis major group. The inferior boundary of the dissection should be the juncture of the latissimus dorsi and the serratus anterior muscles.

The contents within these boundaries should be completely removed with the exception of the long thoracic nerve and the thorocodorsal nerve which should be identified during the dissection and preserved throughout. As stated, the pectoralis minor muscle may be divided or sacrificed with the specimen at the discretion of the surgeon. The preferable approach to the axilla is through a horizontal incision in the line of the skin crease, 3 or 4 cm below the apex of the skin fold of the axilla.

Inguinal Lymphadenectomy (Superficial Groin Dissection):

A superficial groin (femoral / inguinal) node dissection should be performed by excising all of the nodes inferior to the inguinal ligament and bounded by the medial border of the sartorius muscle in the lateral border of the adductor magnus muscle. The fatty and lymphatic tissues should be dissected carefully off of the femoral vessels and nerves all the way up to the inguinal canal and for 2-3 cm superior to the inguinal ligament (the superficial subcutaneous tissue cranial to the exit of the femoral canal). The saphenous vein is resected to ensure complete excision of the lymphnodes. Transposition of the sartorius muscle should be considered (but is not mandatory) to cover the femoral vessels after complete lymphatic excision. Ideally, this area should be entered through a curvilinear incision staring laterally over the inguinal ligament and curving medially and inferiorly ending over the mid-point of the

adductor magnus muscle. For a superficial groin node dissection, a minimum of 5 nodes must be pathologically investigated.

Deep Groin (External Iliac and Obturator) Node Dissection:

Deep Inguinal and External Iliac Node Dissection can be most easily approached by incising the abdominal wall musculature 3 or 4 cm superior to the inquinal ligament. This incision is taken down through the external oblique, internal oblique and transversus muscles and the surgeon at that point stays extraperitoneally as in the approach to the iliac vessels for renal transplantation. With this approach, the external, internal and common iliac arteries are exposed and the lymphatics coursing among the iliac vessels are excised. The obturator nodes along the obturator nerve and artery should also be resected through this approach. A full combined superficial and deep groin (ilio-inguinal) node dissection is advised in case of when Cloquet's node is positive or if there is other evidence of extended involvement to the deep nodes preoperatively.

7.3.4 Post treatment evaluation

Follow-up will start at week 12 with a CT or PET/CT scan according to the national/center's standard. Patients should be evaluated every 3 months by physical examination and lab testing for up to three years. Every 6 months the patients should be evaluated by CT or PET/CT according to the national/centers' standards.

Subsequent structured follow-up will be according to the current national melanoma guidelines (for example in The Netherlands year 4 and 5 every 6 months, year 6 - 10 once a year).

In case of tumor relapse tumor biopsies (3x14G), PBMC (see Appendix for CIMT protocol, deviations according to own centers' standard protocol are allowed), EDTA blood, and plasma collection will be done.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.4.1 Specific criteria for withdrawal

If after having signed informed consent but before initiation of the treatment, patients do not fulfill the eligibility criteria anymore, patients will be withdrawn from the study and will be replaced by another patient.

7.5 Replacement of individual subjects after withdrawal

Patients that are withdrawn prior to initiation of the treatment and patients that do not fulfill the eligibility criteria will be replaced.

7.6 Follow-up of subjects withdrawn from treatment

Follow-up of patients withdrawn from the study during treatment will continue at least until 31 weeks after the treatment or until the treatment related toxicity has resolved to grade 2 or less (CTCAE 4.03). Thereafter, patients will receive standard follow-up for the disease.

7.7 Premature termination of the study

In case of unexpected toxicity or feasibility (see 5.4) the sponsor will discuss premature termination of the study with the DSMB, the other PIs, the ethical committee, and with BMS.

Table 2. Schedule of assessments[1]	**Screening/BL** week -4 till 0	**Treatment** week 0	week 3	**week 5[2]**	**Pre surgery** week 6	**Post Surgery[3] week 6**	**week 9**	**End of study/ week 12**	**Follow-up[4]**
Informed consent	x								
PA confirmation stage 3 melanoma	x								
Medical history	x								
Physical examination*[5]	x	x	x	x	x		x	x	x
Hematology*[6]	x	x	x	x	x		x	x	x
Chemistry*[7]	x	x	x	x	x		x	x	x
Coagulation: PT/INR and aPTT	x								
beta-HCG pregnancy test[8]	x	x	x	x	x		x	x	
Serology[9]	x								
PET/CT	x							x[#]	x[#]
Brain MRI*	x								
CT neck, chest, abdomen, pelvis	x				x			x[#]	x[#]
ECG*	x								
Adverse events[10]		x	x	x	x		x	x	x
Blood for PBMCs, EDTA[11]	x	x			x	x		x	x[12]
Tumor biopsy	x[13]								x[12]

*additional time points if clinically indicated

1 for treatment schedule see section 6.1

2 Only for patients in arm C

3 during surgery, post tumor removal, collection of 100 ml heparinized blood

4 follow-up every 3 months up to 3 years

5 WHO PS, height, weight, temperature, pulse, blood pressure, complete examination at BL, targeted

6 Hb, white blood cell differentiation and platelet count, Hct

7 LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, glucose, lipase, amylase, TSH, fT4, ACTH, cortisol, LH, FSH, S100, CRP, ESR

according to the national/center's standard

8 only WOCBP

9 HIV, HbsAG, anti-HCV, anti-CMV

10 AEs will be graded according to CTCAE 4.03, SAE reported up to 100 days post last infusion

11 100 ml heparinized blood (for PBMC) + 20 ml EDTA blood (for TEP and circulating DNA); during screening, at baseline, week 6 (pre- and post-surgery), week 12, and at relapse

12 only at relapse

13 Three biopsies taken with a 14g needle during screening and at relapse

8. Safety reporting

8.1.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

- Potential drug induced liver injury (DILI), renal failure, or pneumonitis are also considered an important medical event.
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.
- Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).
-

Potential Drug Induced Liver Injury (DILI)

Potential drug induced liver injury is defined as:

1) AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),

AND

3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

The following hospitalizations are not considered SAEs:

- admissions as per protocol for a planned medical/surgical procedure
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).
- Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

8.2.3 Reporting of SAEs

All Serious Adverse Events (SAE) occurring from registration until 100 days after the last protocol treatment/administration should be documented and reported (the study specific SAE form should be used) within 24 hours immediately to the NKI-AVL Safety Desk

preferably by email to **drugsafety@nki.nl** or by fax: +31 (0)20-5122679; office hours 9 am till 5 pm (CET) Monday to Friday.

The Safety Officer will notify the coordinating investigator immediately of any serious adverse event (as defined above) experienced by a patient. The coordinating investigator will evaluate the SAE and will decide whether the event reported could be related to the protocol treatment and whether it is, both, unexpected and serious (SUSAR, see 8.2.4.).

The NKI-AVL Safety Desk will report the SAE's by email within 24 hours to the subsidizing party BMS Company Global Pharmacovigilance & Epidemiology (GPVE) at worldwide.safety@bms.com.

The safety department of the NKI-AVL will follow SAEs. Updates on the SAE will be sent to BMS GPVE similarly, within 24 hours of receipt.

In addition, serious adverse events will be reported to the METC and the competent authority in each country once yearly, as described in the section 8.3. SAE's will not be reported through the web portal ToetsingOnline to the METC in the Netherlands.

Serious adverse events occurring more than 100 days after the last study medication will NOT be reported unless the investigator feels that the study drug or a protocol procedure may have caused the event.

Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as 'serious', UNLESS at least one of the following exceptions are met:

• the admission is pre-planned (e.g. elective or scheduled surgery, documented in the patient's file);

• hospitalization for technical (e.g. study drug administration), practical or social reasons, in the absence of a serious adverse event.

Common toxicities observed for progressive disease and events secondary to progressive disease are generally excluded from reporting. However, in cases where the specificity or severity of an event is not consistent with the risk information, the event should be reported.

All SAE reports will be filed in the Investigator Study File.

In case the SAE is unexpected a SUSAR will be reported (see section 8.2.4).

8.2.4 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);

2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;

3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:

- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

– SUSARs that have arisen in the clinical trial that was assessed by the METC;

– SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs will to be reported till 100 days after discontinuation of the study drug.

8.5 Data Safety Monitoring Board (DSMB) / Safety Committee

A DSMB will be installed (see also DSMB charter). The DSMB will meet for interim and final analysis (after 39 patients and 90 patients have been included). The DSMB will evaluate the safety of the treatment arms and decide whether the arms shall be continued (see 4.5). The DSMB will also advise on which scheme(s) should be considered to be investigated in a phase 3 trial.

9. STATISTICAL ANALYSIS

See chapter 4.4 Sample size calculation.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki, (Declaration of Helsinki, 59th WMA General Assembly, Seoul, October 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice

(ref:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf).

The protocol must be approved by the CCMO and competent authority (CA) in the Netherlands and the local METCs of the participating centers.

10.2 Recruitment and consent

Informed consent (IC)

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements.

The IC procedure will conform to the ICH guidelines on Good Clinical Practice. This implies that "the written IC form will be signed and personally dated by the patient or by the patient's legally acceptable representative".

Recruitment

It is the responsibility of the investigator to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding her rights, the objective and procedures of the trial and the possible risks involved. Confidentiality-related information will be provided. The written patient information must be given to each patient. Patients will be given sufficient time for consideration. An independent physician will be available in

accordance with the requirements of the national law. It is the responsibility of the investigator to obtain signed informed consent from every patient prior to the start of any study related procedure. The written patient information is part of the documentation reviewed by the MEC mentioned. The patient information letter and informed consent form are attached as a separate document.

10.3 Benefits and risks assessment, group relatedness

See pages 12 and 23

10.4 Compensation for injury

In accordance with Statutory Instrument 1031 and amendments section 15 (5i, j), the EU Clinical Trials Directive 2000/20/EC Article 3 (2f), and the Australian Clinical Trials Directive provision by participating each center is to be made for:

• The indemnity or compensation in the event of injury or death attributable to the clinical trial; and

• Insurance or indemnity to cover the liability of the Investigator or Sponsor.

Therefore each participating institution will indemnify the Investigators from all and any claims arising out of this study except for their negligence or malpractice and providing that the study is conducted according to the standards established by the protocol.

Each participating center is responsible and will ensure sufficient insurance coverage of their patients according to their national standards.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.5 Incentives (if applicable)

Not applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Subject identification

Following enrolment a patient sequential identification number or code will be allocated to the patients. This number will be used for identification of the patients Data and patient material will be handled confidentially and if possible anonymously. When it is necessary to trace data or material to an individual subject, we will use a subject identification number list to link the data or material to the subject. The code is based on the sequence of enrolment combined with month-year of the patients' birth date: **# MMYYYY**. The key to the code will be safeguarded by the coordinating investigator and the principle investigator of each center. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP) and to the protection act of each individual country.

11.2 Randomization of the patients

Patient randomization will only be accepted from authorized investigators or through their authorized data manager or authorized staff member. A patient can be randomized only after verification of eligibility. During the randomization procedure eligibility criteria and items of patients' informed consent are checked. Randomization will be done online using the ALEA randomization software package. An automatic confirmation letter will be sent by e-mail to the participating investigator. If necessary, the Trial Office of the AVL can be contacted to assist with the randomization (during CET work hours: 9 am until 5 pm).

11.3 Storage of patient material

Patient material will be initially stored initially at the participating centers, and will be transferred in batches to the NKI-AvL. Here, the tissue will be stored at the core facility molecular pathology and biobanking (CFMPB). The peripheral blood monocytes will be generated from peripheral blood by ficoll according the CIMT protocol (see Appendix, deviations, if not major, are allowed), and stored in liquid nitrogen at the immunology division of the NKI-AVL. Material that is not used for current translational research will be stored for up to 15 years after end of study.

11.4 Data management

All data that are relevant for the study will be collected on eCRFs developed by the datacenter of the AVL. The completed eCRFs must be reviewed and signed by the principal investigator or sub-investigator.

11.5 Monitoring and Quality Assurance

Source data verification of the CRFs and check of the Investigator Study File documents will be performed by the clinical research monitor, according to the procedures described in the Monitor Plan.

11.6 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the investigator.

11.7 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.8 End of study report

The investigator of each center will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.9 Public disclosure and publication policy

Prior to initiation, the study will be submitted to the www.clinical.trials.gov, which is a recognized and accepted by the World Health Organization and International Committee of Medical Journal Editors (ICMJE) and to the NCI's PDQ® Cancer Clinical Trials Registry. All the results will officially be published.

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13. APPENDICES – CIMT PBMC PROTOCOL, THROMBOCYTE, PLASMA COLLECTION, AND SAFETY ALGORITHMS

13.1 Appendix A - PBMC isolation

1. SCOPE

These procedures should be performed by site staff experienced in the isolation and cryopreservation of **PBMCs and granulocytes** in patient studies.

The purpose of this work protocol (WP) is to provide guidance on handling of blood samples for PBMC isolation by density gradient centrifugation and cryopreservation. The WP is intended to ensure consistently high sample quality.

2. SAMPLE

Heparinized whole blood.

3. EQUIPMENT AND MATERIALS

Equipment:

Laminar flow Cabinet

Table Centrifuge

Materials:

Products	Description	Vendor/ Number
50 ml tubes	Polypropylene disposable conical tubes (blue cap)	BD Falcon # 352070
96 well plate	polypropylene microplate 96 well U shape	Greiner Bio-one #: 650185
Pipettes	pipette single 0.5 -10 µl, 10 - 100 µl, 100 – 1000 µl	VWR # resp. 613-2517, 613-2525, 613-2526
Pipette Boy	accu-jet® pro	VWR # 612-2625
Pipette tips	Pipette tip 10 and 200, 1000 µl filter tips	Thermo scientific # 9400 303 and Greiner Bio-one # 780288, 740288

Products	Description	Vendor/ Number
Pipettes (disposable individually wrapped)	5 ml, 10 ml, 25 ml, nonpyrogenic	BD Falcon # resp. 357543, 357551, 357525
Nalgene 2-propanol-based freezing container	Nalgene cryofreezing container, “Mr. Frosty”. For control rate freezing, stored at 2 - 8 ºC fridge	Nalgene # 5100-0001
Cryogenic Freezing vials	1.5 ml polyethylene CryoTubes™, with screw cap, NUNC	NUNC # 377267
Bürker hemocytometer	Brightline counting chamber,	VWR # 631-1124
Turk solution	For assessment of white blood cell count, diluted 1:10 in cell suspension,	Carolina Biological Supply company # 89-7213
Dulbecco’s Phosphate Buffered Saline without Ca and Mg (PBS)	1x D-PBS sterile, in 500 ml bottle, unopened bottles stored in 2 - 8 ºC fridge	GIBCO Life Technologies #14190-144
Ficoll-Paque Plus™	Mononuclear cell separation solution, aliquots in 50 ML tubes, stored at 2 - 8 ºC	GE Healthcare Biosciences AB #17-1440-03
Dimethyl sulphoxide (DMSO)	1 L bottle,	Sigma Aldrich # 34943
Penicillin/Streptomycin (pen/strep)	Liquid, (500X), used conc. 100 U/ml penicillin and 100 µg/ml streptomycin. Stored in 1 ml aliquots in freezer -20 ºC,	Roche # 11074440001
RPMI 1640 Medium (RPMI)	RPMI contains, L-glutamine and HEPES 25mM, in 500 ml bottles, stored at 2-8 ºC	GIBCO # 52400-025
Fetal bovine serum	Heat Inactivated FBS, stored	GIBCO # 10500-064

Products	Description	Vendor/ Number
(FBS)	in 40 ml aliquots at -20℃	
Freezing medium	20% DMSO + 80% FBS mixture, freshly made or aliquots are stored at 2-8 ℃	Sigma # 34943 GIBCO # 10500-064
2-propanol	Use for the Nalgene cryofreezing container	Sigma Aldrich # 33539
DNA isolation buffer	100 mM NACl, 25 mM Tris pH 8, 10 mM EDTA, dissolved in distilled water, stored at 2-8 ℃	Merck # K23313400 (NaCl) Biosolve # 20092391 (Tris) Aldrich # E2628-2 (EDTA)
Erythrocyte lysisbuffer	NH_4Cl 0.155M / $KHCO_3$ 0.01M / Na_2EDTA 0.1M, dissolved in distilled water, pH 7.4, stored at 2-8 ℃,	Merck # 1011430500 (NH_4Cl) Merck # 10148540500 ($KHCO_3$) Merck # 324503 (Na_2EDTA)

4. REAGENT PREPARATION

Note: All solutions should be clearly labelled for contents, preparation date, expiration date, and should be stored at the proper temperature and light conditions.

Make a sufficient amount of complete RPMI (**cRPMI)**, **used for the S134, S138 and S177 studies**

cRPMI; 500 ml RPMI 1640 supplemented with: 50 ml FBS (final concentration of 10% FBS) and 1 ml pen/strep stock solution (with a final concentration of 100 U/ml penicillin and 100 µg/ml streptomycin).

Freezing medium: use aliquoted FBS and mix this with 10% DMSO. Freshly made freezing medium or aliquots are stored at 2-8 ℃.

5. PROCEDURE FOR PBMC AND GRANULOCYTE ISOLATION

1. Label all tubes with the appropriate identification patient code.
2. Transfer the heparinized blood to 50 ml tubes.
3. Note the total amount of blood.

4. Wash each blood tube with 5.0 ml sterile 1XPBS, with use of a disposable individually wrapped pipette and transfer to the 50ml. The 50 ml tubes are maximal filled up to 35 ml with diluted blood
5. Pipette 10 ml of Ficoll-Paque carefully and very slowly under the pre-diluted blood at the bottom of the tube and withdraw the pipette very slowly. Centrifugate 20 minutes at 400g (=1900 rpm for centrifuge Hettich Rotanta/AP # IV, lab B3.015) and RT in a swinging bucket rotor. **Switch off brakes of the centrifuge**
6. Harvest carefully the interlayer (PBMC) by means of a 10 ml pipette and transfer into a new 50 ml tube
7. Wash the cells with sterile PBS and centrifugate for 12 minutes at 160g (=1200 rpm centrifuge Hettich Rotanta/AP # IV , lab B3.015)

In case the processed blood is the screening sample of a patient then also granulocytes have to be isolated. This procedure is given below. If not necessary continue with point 10-14 and 21

8. Harvest carefully the top layer, the first 4-5 ml of the red blood cell layer (granulocytes) and transfer this into a new 50 ml tube
9. Add 40 ml erythrocyte-lysisbuffer to the granulocyte / erythrocyte cell suspension and incubate this on a roller bar for 15 minutes at room temperature

10. Meanwhile decant the supernatant of the spin down PBMC and resuspend cell pellet.
11. Add 10 ml erythrocyte-lysisbuffer to the PBMC cell suspension and incubate this on a roller bar for 10 minutes at room temperature
12. Wash the PBMC by adding 40 ml PBS and centrifugate for 5 minutes at 400g (=1600 rpm centrifuge Hettich Rotanta/AP # IV at the ML2 lab)
13. Decant the supernatant of the spin down PBMC and resuspend cell pellet.
14. Wash the PBMC by adding 40 ml PBS and centrifugate for 5 minutes at 400g (=1600 rpm centrifuge Hettich Rotanta/AP # IV at the ML2 lab) Decant supernatant and resuspend cell pellet with 2.5 ml of cRPMI

15. Centrifugate the granulocyte / erythrocyte - lysis cell suspension for 5 minutes at 400g
16. Decant supernatant and wash with 30 ml PBS by centrifugate for 5 minutes at 400g
17. Decant supernatant and resuspend pellet in 0.5 ml DNA isolation buffer
18. Transfer 0.5 ml of this cell suspension into a 2.0 ml eppendorf tube
19. Label the tube by marking it with: <'Patient Study_CODE'>, <'Granulocytes'>, and the <'Sample Date'>

20. Place the vials in the freezer -80 ℃

21. Count the isolated PBMC in suspension for freezing down procedure

6. CELL COUNTING

Lymphocytes will be counted using a Bürker hemocytometer

Cell counting with the Bürker hemocytometer:

1. Resuspend cells thoroughly, take 10 µl of the cell suspension and combine with 90 µl of Turk solution (final dilution 1:10) in a well of a U- tube shaped 96-well plate
2. Mix the cell suspension and load it onto the Bürker hemocytometer by placing the tip of pipette to the space between the groove of the Bürker hemocytometer and the cover slip
3. Count cells in 25 squares of the Bürker hemocytometer. Count all the cells within the triple line boundary; cells can be touching this boundary to the left or the upper line, but not to the right or the lower line and should not cross the boundary
4. The number of counts multiplied by 10^4 will give the number of cells per ml of the sample. To obtain total cell number, multiply the number of cells/ml x total volume of cell suspension
5. Clean the Bürker hemocytometer and cover slip with 70% ethanol

7. FURTHER PROCESSING OF THE ISOLATED CELLS

The freshly isolated PBMC will be divided into samples of **10 – 12 * x10^6 PBMC/ ampule.**

Bring the cell suspensions to the right concentration. If the cell concentration is too high to freeze down procedure, change the volume of the cell suspension by adding extra cRPMI according to the study. On the other side in case of a too low concentration, spin down the cells again for 5 minutes at 400g, discard the supernatant and resuspend the cells in the right volume cRPMI.

Put cell suspension(s) and freezing medium on ice, before starting the freezing procedure by adding the freezing medium.

8. CRYOPRESERVATION OF PBMC

1. Nalgene freezing container, “Mr. Frosty” should be filled with 2-propanol. Before use the container should be stored at 4°C for at least 1 hour
2. Label the cryovials by marking it with: <‘Patient Study_CODE’>, <’PBMC’>, <’ additional number code (‘1’, ‘2’, ‘3’, …) for each vial>, and the <’Sample Date’>
3. Resuspend the PBMC pellet in cold medium cRPMI at a concentration as described above in a 50 ml tube
4. Place the 50 ml tube with cell suspension and the freezing medium mixture (20% DMSO/ 80% FBS) on ice preferably for 15 minutes or longer
5. Start freezing process while gently swirling the tube and adding drop wise ice cold freezing medium to exactly double the volume of the cell suspension
6. Mix well and aliquot this PBMC suspension into the pre-labeled cryovials: 1 ml into each 1.5 ml vial
7. Immediately place the cryovials into pre-cooled Nalgene cryofreezing container, “Mr. Frosty”
8. Transfer the cryofreezing container into a –80°C freezer for a minimum of 12 hours. Do not open the container during this period in order to prevent shaking the samples or fluctuation of the freezers temperature
9. After a minimum of 12 hours and a maximum of 3 days, transfer the cryovials into liquid nitrogen tanks
10. Record the number of vials and the respective cell numbers in a sample recovery form and a Excel folder

9. SAMPLE RECORDING

For sample recording the patient’s study code plus year of birth will be used. Each ampule get an additional number code (“-1”, “-2”, “-3”,…).

13.2 Appendix B – Tumor educated platelets (TEP) and Plasma (for circulating DNA)

EDTA tube – 10 mL blood

Process within 8 hours of collection
Always wear gloves during processing
Use sterile material

1. Centrifuge at room temperature – 120g, 20min
2. Gently pipet the platelet-rich plasma (PRP) in a 50 ml sterile centrifuge (falcon) tube using a 1000 µL pipet: Make sure you do not take any white blood cells (WBC).
3. Centrifuge the PRP at room temperature – 380g, 20min
4. Remove all supernatant (EDTA plasma) into 3x 2 mL Eppendorf tubes. (continue for plasma isolation at step 9)
5. Remove any left-over supernatant using a 50 µL pipet and
6. Add 50 µL RNA*later* to the pellet, and carefully resuspend the platelets by gently pipetting the pellet several times using a 100 µL pipet.
7. Divide the thrombocytes into 2 cryo vials
8. Store for 24 hours at 4°C and then freeze at -80°C
9. Centrifuge the tubes with EDTA plasma for 10 min at maximum speed (20.000g)
10. Pour the plasma into 3 cryo vials and store at -80°C

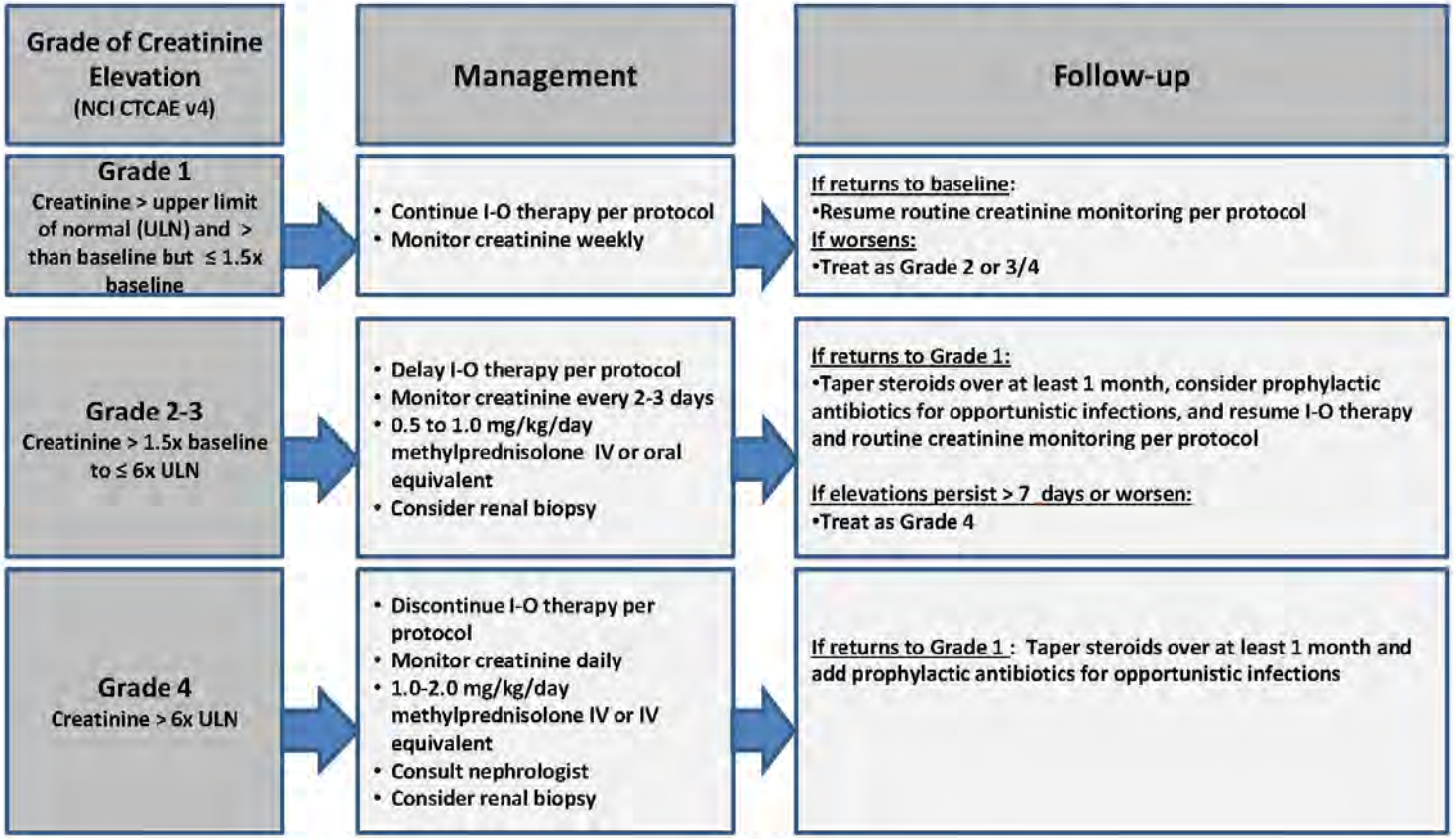
13.3 Appendix D – Safety Algorithms

Please see also Chapters 6.7 - 6.9 for study specific treatment delay and discontinuation rules.

Please be aware of intensified colitis management in this trial (if one week after infliximab 5mg/kg no full recovery, then infliximab 10mg/kg, if no signs of recovery within 2 days, then proceed to tacrolimus according to hepatic adverse event management)

Renal Adverse Event Management Algorithm

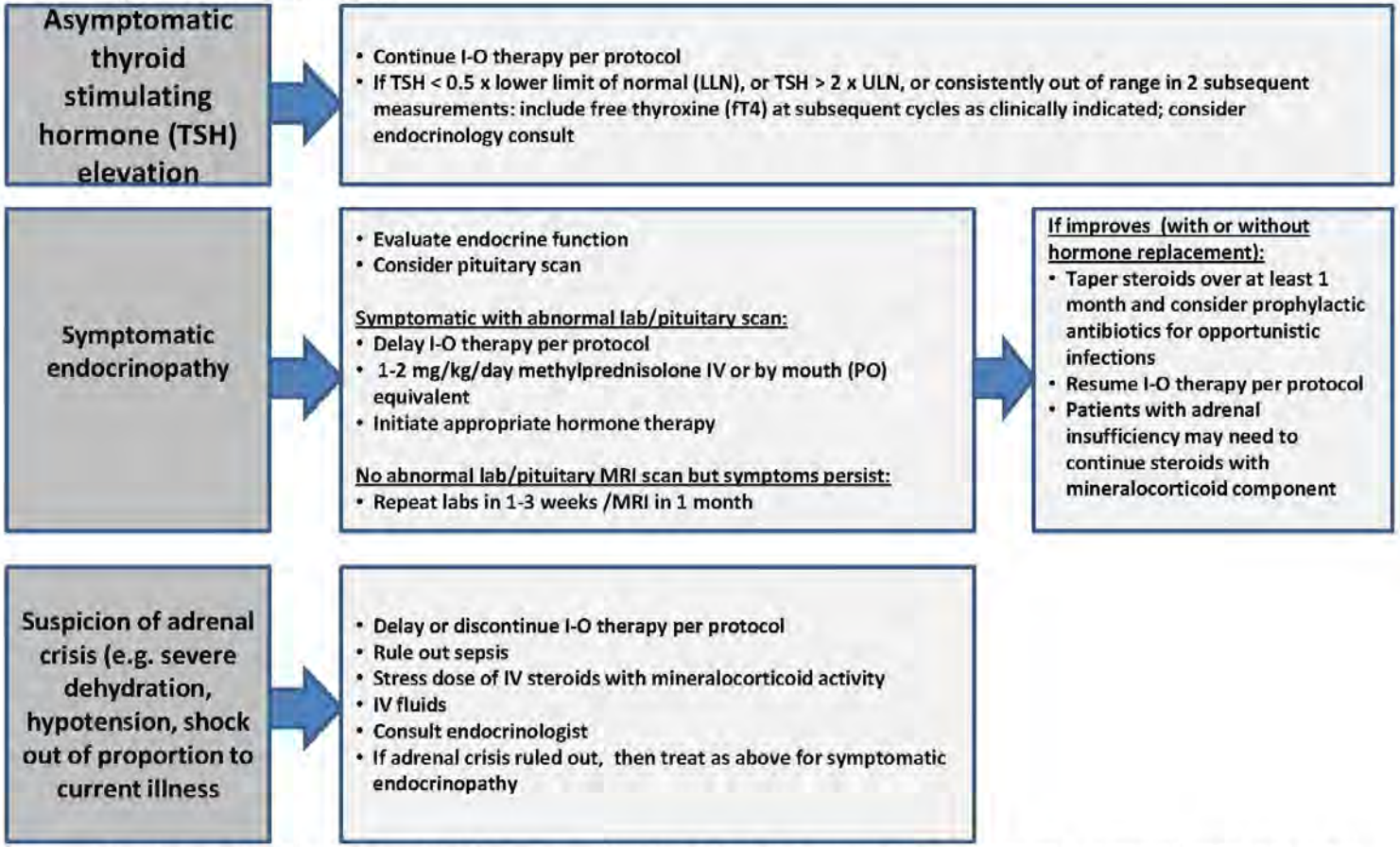
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Endocrinopathy Management Algorithm

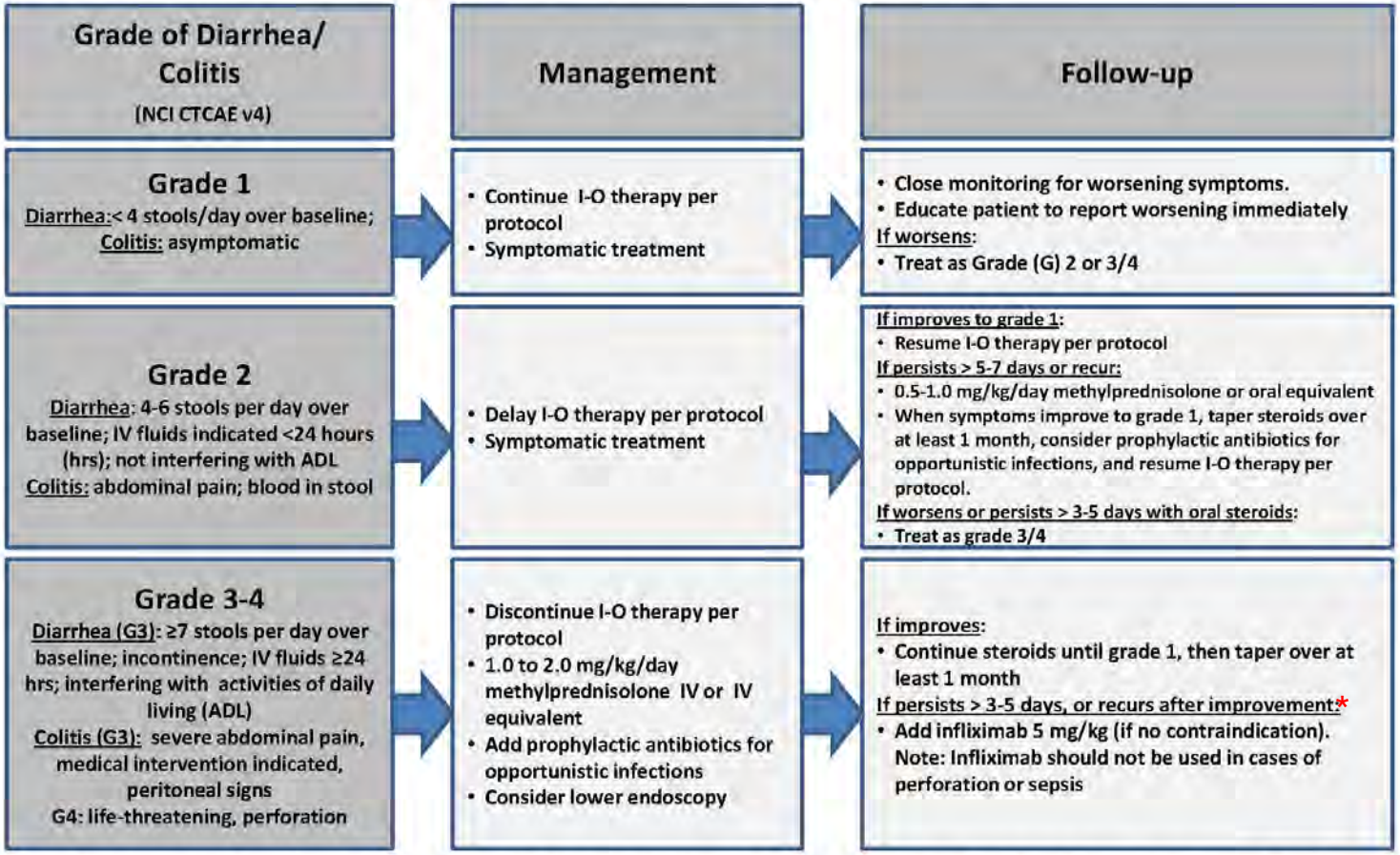
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

* if one week after infliximab 5mg/kg no full recovery, then infliximab one infusion 10mg/kg, if no signs of recovery within 2 days, then proceed to tacrolimus according to hepatic adverse event management

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.**

****The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.**

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

Grade of Neurological Toxicity (NCI CTCAE v4)	Management	Follow-up
Grade 1 Asymptomatic or mild symptoms; Intervention not indicated	• Continue I-O therapy per protocol	Continue to monitor the patient. If worsens: • Treat as Grade 2 or 3-4
Grade 2 Moderate symptoms; Limiting instrumental ADL	• Delay I-O therapy per protocol • Treat symptoms per local guidelines • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent	If improves to baseline: •Resume I-O therapy per protocol when improved to baseline If worsens: • Treat as Grade 3-4
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	• Discontinue I-O therapy per protocol • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: • Taper steroids over at least 1 month If worsens or atypical presentation: • Consider IVIG or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

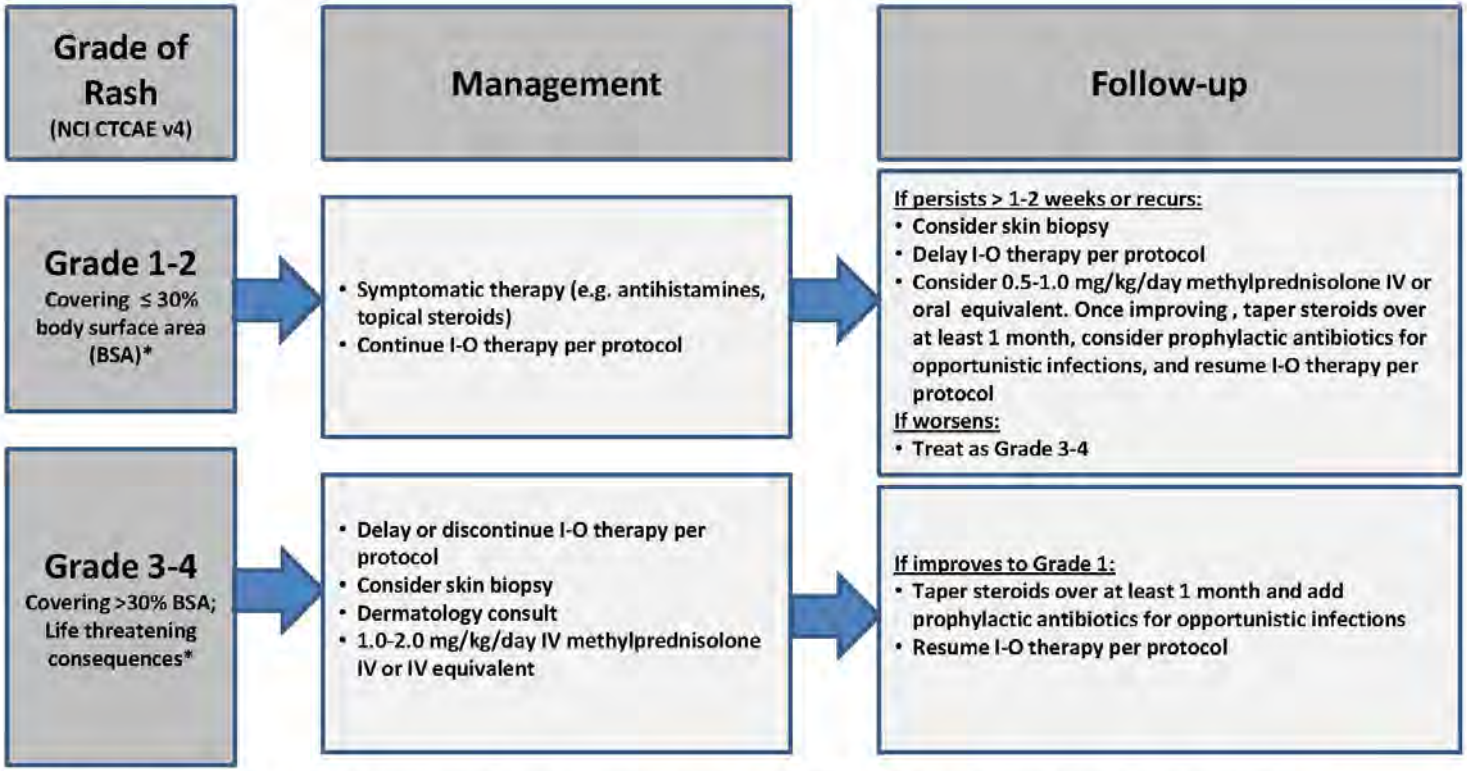
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

Feasibility Study to Identify the Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN) in resectable stage III melanoma patients

Version 4/dd 21-05-2015

PROTOCOL TITLE

Feasibility Study to Identify the Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN) in resectable stage III melanoma patients

Protocol ID	**CA209-278/N14OPC**
Short title	**Optimal Adjuvant Combination Scheme of Ipilimumab and Nivolumab (OpACIN)**
EudraCT number	***2014-004764-38***
Version	**4**
Date	**21 May 2015**
Coordinating investigator/project leader	***Dr. C.U. Blank***
Principal investigator(s) (in Dutch: hoofdonderzoeker/ uitvoerder)	***Dr. C.U. Blank***
Sponsor (in Dutch: verrichter/opdrachtgever)	***Antoni van Leeuwenhoek Hospital***
Subsidising party	***Bristol-Myers Squibb***
Independent expert (s)	***Dr. M. vd Heuvel***
Laboratory sites	***Not applicable.***
Pharmacy	***Pharmacy Antoni van Leeuwenhoek Hospital***

PROTOCOL SIGNATURE SHEET

Name	**Signature**	**Date**
Sponsor or legal representative: ***Prof. dr. E. Voest*** **Head of Department:** ***Prof. dr. J.B.A.G. Haanen***		
[Coordinating Investigator/Project leader/Principal Investigator]: ***Dr. C.U. Blank***		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
AE	**Adverse Event**
AR	**Adverse Reaction**
BMS	**Bristol Myers Squibb**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CTLA-4	**cytotoxic T-lymphocyte-associated protein 4**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
IB	**Investigator's Brochure**
IC	**Informed Consent**
irAE	**Immune related adverse event**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
PD-1	**Programmed death receptor-1**
PBL	**Peripheral Blood Lymphocytes**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but**

referred to as a subsidising party.

SUSAR **Suspected Unexpected Serious Adverse Reaction**

Wbp **Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**

WMO **Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: T cell checkpoint blockade by anti-CTLA and/or anti-PD1 is currently the most promising therapy in late stage melanoma to induce long-term benefit or even cure. Particularly the combination of ipilimumab and nivolumab induces high response rates and promising response depth. This raises the question whether ipilimumab and/or nivolumab could also become standard therapy in adjuvant treatment of melanoma. Ipilimumab has been tested with promising results in stage III melanoma [1], however due to short follow-up time (median 3 years) up-to now, no melanoma specific survival (MSS) data could be analyzed or reported. These results are expected by 2016.

In contrast to chemotherapeutic approaches, immunotherapeutic approaches depend on sufficient activation of the immune system. To become fully activated, T cells require two signals. The first signal is provided by the interaction of the (tumor-) antigen presented in the major histocompatibility complex (MHC) on the antigen-presenting cell (APC) to the T cell receptor (TCR) on the T cell (signal 1). In parallel, a large number of co-inhibitory and co-stimulatory interactions – so-called T cell checkpoints - modulate the outcome of the TCR – pMHC interaction. Antibody-based interference with the T cell checkpoints CTLA-4 and PD-1 has been shown to improve tumor-specific T cell responses and to result in a significant clinical benefit in patients with melanoma and other cancers.

The notion that T cell checkpoint inhibition is of greatest value at the moment of TCR triggering has potentially significant consequences for the use of checkpoint targeting antibodies as adjuvant therapies. Specifically, as the amount of antigen that can provide this signal 1 will correlate with tumor load, we postulate, that adjuvant immunotherapy will work most efficiently, when adjuvant treatment is initiated prior to surgery.

Objectives:

Primary objective: To determine safety, feasibility, and the immune-activating capacity of short-term combined neo-adjuvant and adjuvant ipilimumab + nivolumab.

Secondary objectives: To determine relapse free survival (RFS), any late adverse events, pharmacokinetics/pharmacodynamics, and the correlation between RFS and changes in neo-antigen specific T cell response

Study design:

This is a two-arm Phase 1b feasibility trial consisting of 20 patients receiving the combination of ipilimumab+nivolumab, either adjuvant, or split neo-adjuvant and adjuvant.

Study population:

Resectable stage III melanoma patients with palpable lymph node metastases (and no history or active in-transit metastases within the last 6 months), naïve for CTLA-4/PD-1/PD-L1 immunotherapy, and more than 18 years old.

Intervention:

Resectable stage III melanoma patients with palpable lymph node metastases, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, will be treated either post-surgery for 12 weeks with the combination of ipilimumab+nivolumab or in a split design for 6 weeks upfront surgery and for 6 weeks post-surgery (10 patients per arm, Figure 1).

Medicine tested: Ipilimumab 3 mg/kg q3wks, Nivolumab 1 mg/kg q3wks

Lab testing (incl. PBMC, serum collection) will be performed during screening, at baseline, direct post-surgery, at the indicated time points until week 18, and subsequently every three months.

Tumor biopsies/material preservation is required at baseline and during surgery.

CT scans will be required at baseline, week 6 (only neo-adjuvant arm), week 18, and subsequently every 3 months up to 3 years.

Another PBMC, serum collection and tumor biopsies will be performed at the timepoint of relapse.

Figure 1

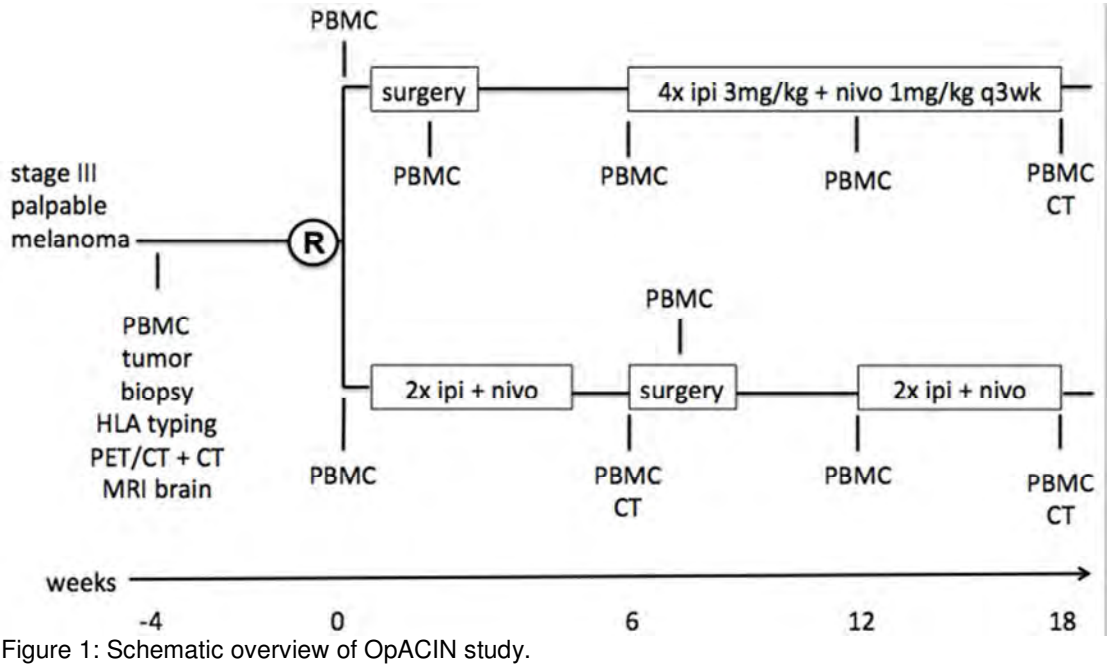

Figure 1: Schematic overview of OpACIN study.

Main study parameters/endpoints:

Primary endpoints:

- Primary readout will be the alteration in magnitude or breadth of the neo-antigen specific T cell response in the time interval pre- to post-adjuvant therapy in peripheral blood. To this purpose the immunogenic mutational load of each patient's melanoma will be determined by DNA and RNA sequencing from baseline biopsies (3x14g, 5ug tumor DNA). Proteasomal degradation and peptide presentation in HLA will be predicted in silico. MHC-tetramer staining containing the predicted peptides will be done as described before [2].

In addition, we will analyze the effect of therapy on intratumoral T cell responses to obtain better insight into the mode of action of therapy. Identified neo-antigen specific T cells will be analyzed with respect to their phenotype and immunologic function (intracellular cytokine staining, lytic function as determined by CD107 staining, and coculture with APC presenting the cognate antigen).

- Safety and feasibility as measured by SUSARs and adherence to the timelines in the study protocol.

Secondary endpoints:

- RFS, as determined according to RECIST 1.1 criteria.
- Rate and type of adverse events and late adverse events
- Correlation between RFS and the delta of magnitude and/or breadth of neo-antigen T cell population
- Pharmacokinetics and pharmacodynamics of nivolumab and ipilimumab comparing the two different treatment arms

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Currently there is no standard adjuvant therapy for stage III melanomas. Post-surgery adjuvant radiotherapy is commonly applied, because it has been shown to marginally improve local disease control, but neither benefit in RFS, nor overall survival (OS), can be achieved in this high-risk patient population [3, 4]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable survival benefit (two out of three meta-analyses found no OS benefit [5-7]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe. Adjuvant systemic immunotherapy with ipilimumab is the only treatment so far to improve RFS in stage III melanoma [1]. However, OS benefit has not

been shown thus far (possible due to short follow-up period) and toxicity was high, mainly when applied longer than four courses of ipilimumab (12 weeks).

Participants of this study will be exposed to two immunotherapeutic agents (ipilimumab and nivolumab) known to induce immune related adverse events at a high percentage when combined together [8]. However, this exposure will be restricted to 4 courses (12 weeks) in total.

In addition patients within this trial cannot be treated with local radiotherapy, which bears the risk of reduced local tumor control. Considering the fact that local irradiation only improves local tumor control, but does not improve RFS or OS, participation in this trial might offer the chance for improved recurrence free survival. This is based on the observation that adjuvant ipilimumab has already shown this benefit in a prospective randomized controlled phase 3 trial in stage III patients and the combination of ipilimumab with nivolumab induces superior response rates as compared to ipilimumab alone in stage IV melanoma [1, 9, 10].

1. INTRODUCTION AND RATIONALE

Background Melanoma

Melanoma is one of the fastest growing malignancies with more than 5,000 new diagnoses per year and almost 800 deaths in 2012 in the Netherlands [Dutch tumor registry (IKNL), www.iknl.nl], and approximately 130,000 new diagnoses worldwide, with 37,000 deaths (WHO, 2012). Surgical resection is the treatment of choice for localized melanoma and frequently results in cure for the early stages (stage 1 and 2), with around 90% long-term survival rate for stage 1 melanoma [11]. However, patients with lymph-node involvement (stage 3), including those detected only by sentinel node biopsy, have a high risk of local and distant relapse after surgery, and 5 year survival drops to 39-70% in this patient group [11]. Specifically, patients with stage IIIB disease [macroscopic / palpable (=non-SN detected)] have a five year survival between 20 – 59%, with certain subgroups (number of involved nodes, extracapsular extension, iliac involvement in groin metastases), which show even worse survival rates of around 5% at 5-years [11-13]. No clinically significant improvement in survival has to date been achieved by adjuvant therapy in this patient group. Adjuvant radiotherapy has been shown to marginally improve local disease control, but neither benefit in RFS, nor in OS, has been achieved in this high-risk patient group [3, 4]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable OS benefit (two out of three meta-analyses found no OS benefit [5-7]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe, and not applied in The Netherlands.

Current standard treatment in melanoma

With the growing understanding of genetic alterations in melanoma and immune modulation by T cell checkpoint molecules, two new therapeutic approaches have become standard therapy in late stage melanoma, both improving overall survival (OS) in randomized trials. Targeted therapy inhibiting the BRAFV600 mutation by selective BRAF inhibitors vemurafenib or dabrafenib significantly improved PFS and OS as compared to “standard” chemotherapy DTIC in randomized phase 3 trials [14, 15]. Combined MAPK pathway targeting by BRAF (dabrafenib respectively vemurafenib) and MEK inhibition (trametinib respectively cobimetinib) improves OS further [16-18]. Therefore, treatment with dabrafenib + trametinib is currently being tested in the adjuvant setting (www.clinicaltrials.gov). However, targeted therapies rarely achieve long-term tumor control and tumor progression is observed after median 6-9 months. Therefore it is questionable whether targeted therapy approaches will improve disease free survival in an adjuvant setting.

T cell checkpoint blockade, e.g. antibody-mediated blockade of CTLA-4 and PD-1/PD-L1, has become the most promising approach in treatment of late stage melanoma. Ipilimumab, a monoclonal antibody targeting CTLA-4, achieved improvement of OS in two randomized trials [10, 19]. While inducing less frequently tumor regression than targeted therapies, long-term benefit from immunotherapy is higher in the responding patients [20]. PD-1 blockade, by e.g. nivolumab or pembrolizumab, induces even higher response rates than CTLA-4 blockade [21, 22]. Furthermore, the deepness of clinical responses (percentage of tumor load reduction) appears further enhanced by the combination of nivolumab and ipilimumab [8, 23]. Because of the profound clinical effects of checkpoint blockade and its ability to induce durable tumor control, immunotherapy seems to be the optimal treatment approach for adjuvant therapy in melanoma. Indeed, adjuvant ipilimumab significantly improved disease-free survival, and this e.g. to a higher absolute percentage than observed for herceptin in breast cancer [1, 24, 25]. To which extent this improved RFS translates into improved OS needs to be awaited. Currently an adjuvant trial comparing ipilimumab versus nivolumab versus ipilimumab plus nivolumab is in preparation.

Rationale for neo-adjuvant plus adjuvant immunotherapy

One major challenge, however, is the observation, that in the recently presented adjuvant trial testing ipilimumab, only 7% of patients completed the full scheme of ipilimumab treatment and maintenance for 3 years [1]. This indicates the need for more efficient and shorter adjuvant immunotherapy schemes, which could induce higher T cell immune responses against melanoma (neo-) antigens and subsequent higher disease-free survival rates.

In contrast to chemotherapeutic approaches or targeted therapy, immunotherapeutic approaches depend on sufficient activation of the immune system. To become fully activated, T cells require two signals [26]. The first signal is provided by the interaction of the (tumor-) antigen presented in the major histocompatibility complex (MHC) on the antigen-presenting cell (APC) with the T cell receptor (TCR) on the T cell (signal 1).

In parallel, a large number of co-inhibitory and co-stimulatory interactions – so-called T cell checkpoints - modulate the outcome of the TCR – pMHC interaction (signal 2) [27]. Antibody-based interference with the T cell checkpoints CTLA-4 and PD-1 has been shown to improve tumor-specific T cell responses and to result in clinical benefit in patients with melanoma and other cancers [10, 21, 28]. T cell checkpoint inhibition (signal 2) is only of value at the moment of TCR triggering (signal 1) [27], which has potentially significant ramifications for the use of checkpoint targeting antibodies as adjuvant therapies. Specifically, as the amount of antigen that can provide this signal 1 will correlate with tumor load, we postulate, that

adjuvant immunotherapy will work most efficiently, when adjuvant treatment is initiated prior to surgery.

To directly address this question, this feasibility and safety study will compare in resectable stage III melanoma patients *neo-adjuvant and adjuvant ipilimumab+nivolumab* short-term treatment (for sake of brevity also referred to as 'neo-adjuvant treatment') against short-term *adjuvant ipilimumab+nivolumab* treatment. Primary readout will be the capacity to induce and broaden T cell responses against melanoma-specific neo-antigens. Secondary endpoint will be RFS. The reason for testing the combination of nivolumab and ipilimumab in the neo-adjuvant plus adjuvant setting is their single treatment efficacy in melanoma (see for further information the investigator brochures ipilimumab and nivolumab) and the improved quality of response observed in stage IV melanoma when combining both [8, 9].

Rationale for Combining Nivolumab with Ipilimumab in Melanoma

Lymphocyte homeostasis is regulated by the differential effects of cytokines and an array of cell surface signaling molecules that affect T cell proliferation. One of the primary molecules responsible for T cell expansion in response to antigenic stimulation is CD28. The interaction of CD28 on the T cell, with CD80 (B7.1) or CD86 (B7.2) on the antigen presenting cell, stimulates T cell proliferation and survival (reviewed in [27]). By necessity, a mechanism for inhibition of antigen stimulated T cells is required following control of the antigenic challenge to maintain the viability of the host. One important regulator of T cell contraction following antigen stimulation is cytotoxic T lymphocyte antigen 4 (CTLA-4)34. CTLA-4 expression on T cells is increased following antigen stimulation and it has a significantly higher affinity for both B7 ligands than does CD28 [29]. CTLA-4 triggering leads to an inhibition of T cell proliferation and thereby helps to control T cell responses. The fact that Ctla-4 knockout mice display a lethal systemic immune hyper-activation demonstrates the important role of CLTA-4 in down-regulation of antigen-specific T cell responses [30].

Ipilimumab is a fully human monoclonal immunoglobulin that binds to CTLA-4 and inhibits its interaction with ligands on antigen presenting cells. Ipilimumab has been approved for use in over 40 countries including the United States in March 2011 and the European Union in July 2011. The safety profile is detailed in the Investigator Brochure. In brief, over 12,700 subjects received ipilimumab and the most common adverse events (AEs) were inflammatory in nature, consistent with ipilimumab's mechanism of action, and were generally medically manageable with topical or systemic immunosuppressants.

Clinical trials with ipilimumab have shown activity in a broad range of tumors. In two phase 3 trials, ipilimumab was the first agent to demonstrate a statistically significant improvement in overall survival in subjects with metastatic melanoma [10, 19]. Monotherapy with ipilimumab

at a dose of 3 mg/kg improved overall survival compared to glycoprotein 100 peptide vaccine in subjects with previously treated metastatic melanoma (10.1 versus 6.4 months, hazard ratio 0.66, p-value 0.003) [10]. In the second study of subjects with treatment naive metastatic melanoma, overall survival was significantly longer in patients receiving dacarbazine with ipilimumab at a dose of 10 mg/kg, than in patients receiving dacarbazine with placebo (11.2 versus 9.1 months, hazard ratio 0.72, p-value < 0.001) [19].

A subsequent clinical trial testing the concurrent combination of nivolumab and ipilimumab resulted in even greater objective response rates than those seen with either agent alone in patients with advanced melanoma [9, 10, 31]. A total of 53 patients received concurrent therapy, resulting in an objective response rate of 40%. Evidence of clinical activity (conventional, unconfirmed, or immune related response or stable disease for ≥ 24 weeks) was observed in 65% of patients. At the maximum doses that were associated with an acceptable level of adverse events, 53% of patients had an objective response, all with tumor reduction of 80% or more. Treatment related adverse events were observed in 93% of subjects, with the most common events being rash (55%), pruritus (47%), fatigue (38%) and diarrhea (34%). Grade 3 or grade 4 treatment related events were noted in 53% of subjects, with the most common events being elevated levels of lipase (13%), aspartate aminotransferase increased (13%) and alanine aminotransferase increased (11%). Six of 28 subjects (21%) had grade 3 or grade 4 treatment related events that were dose-limiting. Eleven subjects (21%) discontinued therapy due to treatment related adverse events. The doses of nivolumab at 3 mg/kg and ipilimumab at 3 mg/kg exceeded the maximum tolerated dose due to the occurrence of asymptomatic grade 3 or grade 4 elevated levels of lipase that persisted for ≥ three weeks in three of six subjects. Doses of nivolumab at 1 mg/kg and ipilimumab at 3 mg/kg (n = 17 subjects), in addition to nivolumab at 3 mg/kg and ipilimumab at 1 mg/kg (n = 16 subjects) were both tolerated. Additional clinical experience with over 250 subjects (studies CA209004 and CA209012) has shown that both combination regimens are tolerated in subjects with solid tumors. In patients with advanced melanoma, concurrent treatment with the combination of nivolumab and ipilimumab results in rapid and deep regression of tumors in a substantial proportion of subjects, when compared to either agent alone. Even though approximately 50% of subjects on the combination melanoma study developed grade 3 or grade 4 adverse events, the safety profile was generally manageable, suggesting an acceptable risk to benefit ratio [8, 9].

Background translational research

Tumors such as melanoma and NSCLC that are responsive to T cell checkpoint blockade are characterized by large numbers of mutations, and such mutations can lead to the

presentation of 'neo-antigens' that are foreign to the T cell-based immune system [32]. It was postulated already many years ago that T cell recognition of neo-antigens would form an important ingredient in T cell-based tumor recognition. However, the fact that the vast majority of the mutations within human tumors are patient-specific precluded the efficient analysis of neo-antigen specific T cell immunity until recently. Importantly, through the development of cancer exome sequencing and high throughput immunomonitoring technology (see below), it is now feasible to analyze neo-antigen specific T cell responses in individual patients and the effect of therapy on such responses. From these analyses we have learned that 1). CD8 T cell recognition is common in melanoma (observed in six out of eight patients analyzed; [2] and van Buuren et al., unpublished). 2). neo-antigen specific T cell responses can be increased upon ipilimumab treatment [2], and 3). neo-antigen specific T cell responses can be induced or increased to detectable levels upon nivolumab treatment (Kvistborg et al., unpublished). The ability to monitor the development/ enhancement of neo-antigen-specific T cell responses in patients undergoing treatment provides a unique opportunity to compare the effectiveness of different treatment strategies, in particular in situations (as is the case here) in which a significant clinical readout is only available after long periods of time and with large numbers of patients. Furthermore, by comparing intrapatient changes in neo-antigen specific T cell responses upon therapy, ability to pick up differences is highly relative to a setting in which clinically only interpatient comparisons are feasible.

To achieve the monitoring of neo-antigen specific T cell responses, the repertoire of mutations present within the melanoma of a patient is first analyzed by exome sequencing, using peripheral blood as reference material. In addition RNAseq is used to filter for expressed genes. With the list of patient-specific mutations in expressed genes in hand, potential neo-epitopes are predicted using an algorithm that takes into account predicted proteasomal processing, binding affinity for the HLA class I alleles carried by the patient, and 'dissimilarity to self'. This results in a list of potential neo-epitopes that is synthesized by classical peptide synthesis. Resultant peptides are loaded onto the relevant recombinant HLA class I alleles, using the ligand exchange technology developed by NKI [33]. Subsequently, these peptide HLA complexes are converted into multimers, labeled with fluorochromes and used for highly multiplexed T cell detection (combinatorial coding technology, also developed by NKI). This technology has successfully been used to identify neo-antigen specific T cell responses in patient material [2], and has a very high sensitivity/ reproducibility (Kvistborg et al. Science Translational Medicine, accepted for publication). All identified T cell responses are routinely confirmed in an independent analysis.

This analysis will be performed on pre- and post-treatment peripheral blood samples of patients in both treatment arms, in order to assess how the two treatments influence neo-antigen specific T cell reactivity.

Risk benefit discussion

Currently there is no standard adjuvant therapy for stage III melanoma. Post-surgery adjuvant radiotherapy is commonly applied, because it has been shown to marginally improve local disease control, but no benefit in relapse-free survival, nor overall survival has been achieved in this high-risk population [3, 4]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable survival benefit (two out of three meta-analyses found no OS benefit [5-7]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe. Adjuvant systemic immunotherapy in form of ipilimumab has been tested with promising results in stage III melanoma [1], however overall survival benefit has not been reached thus far and toxicity was high, mainly when applied longer than the four courses aimed for here.

Participants of this study will be exposed to two immunotherapeutic agents (ipilimumab, nivolumab) known to induce immune related adverse events at a high percentage when combined together. In the phase 1 trial testing the combination of ipilimumab+nivolumab 98% of the 53 patients experienced adverse events (of any grade). 93% of adverse events were reported as treatment related, the most common ones being rash (55%), pruritus (47%), fatigue (38%), and diarrhea (34%). Treatment related grade 3 and 4 adverse events were observed in 53%, the most common ones being lipase elevation (13%), AST (13%) and ALT (11%) elevation. SAEs were observed in 49%, including hepatic, gastro-intestinal, renal toxicity and more rarely pneumonitis and uveitis. In analogy to the ipilimumab adjuvant trial one can expect these percentages to be even higher when applying ipilimumab+nivolumab adjuvant [1, 10].

In addition, without local radiotherapy the patients treated within this trial with systemic immunotherapy bears the risk of reduced local tumor control. Argument against this is the chance of improved RFS, based on the observation that single ipilimumab improved RFS in a phase 3 trial, and that the combination of ipilimumab+nivolumab induces superior response rates as compared to ipilimumab alone in stage 4 melanoma [1, 9, 10].

Finally, post-surgery recovery could be altered theoretically when applying neo-adjuvant ipilimumab+nivolumab. Currently there are no data supporting this risk. Related to this, a recent analysis of patients undergoing surgery during ipilimumab treatment revealed no complications [34].

2. OBJECTIVES

Primary objectives:

To determine safety, feasibility, and the immune-activating capacity of short-term combined neo-adjuvant and adjuvant ipilimumab + nivolumab.

Primary endpoints:

- Primary readout will be the alteration in magnitude or breadth of the neo-antigen specific T cell response in the time interval pre- to post-adjuvant therapy in peripheral blood. To this purpose, the immunogenic mutational load of each patient's melanoma will be determined by DNA and RNA sequencing from baseline biopsies. Proteasomal degradation and peptide presentation in HLA will be predicted in silico. MHC-tetramer staining containing the predicted peptides will be done as described before [2]. The changes of magnitude and breadth of neoantigen-specific T cell responses will be compared between the two arms.

- In addition, we will analyze the effect of therapy on intratumoral T cell responses to obtain better insight into the mode of action of therapy. Identified neo-antigen specific T cells will be analyzed with respect to their phenotype and immunologic function (intracellular cytokine staining, lytic function as determined by CD107 staining, and coculture with APC presenting the cognate antigen).

- Safety and feasibility as measured by SUSARs and adherence to the timelines in the study protocol.

Secondary objectives:

To determine relapse free survival according to RECIST 1.1

Identification of adverse events

To identify correlations between RFS and changes in neo-antigen specific T cell response

To determine pharmacokinetics and pharmacodynamics of nivolumab and ipilimumab within the two study arms

Secondary endpoints:

RFS, will be determined according to RECIST 1.1 criteria.

Rate and type of adverse events and late adverse events

Correlation between RFS and the delta of magnitude and/or breadth of neo-antigen T cell population

Differences of pharmacokinetics (serum antibody concentrations), as determined by enzyme-linked immunosorbent assay (ELISA), and pharmacodynamics, as determined by PD-1 and CTLA-4 receptor occupancy on PBL, between the two study arms

3. STUDY DESIGN

Resectable stage III melanoma patients, no history or active in-transit metastases within the last 6 months, and being naïve for CTLA-4/PD-1/PD-L1 immunotherapy, will be treated either post-surgery for 12 weeks with the combination of ipilimumab+nivolumab (Arm A) or in a split design 6 weeks upfront surgery and 6 weeks post-surgery (Arm B), each 10 patients per arm (Figure 1).

Figure 1

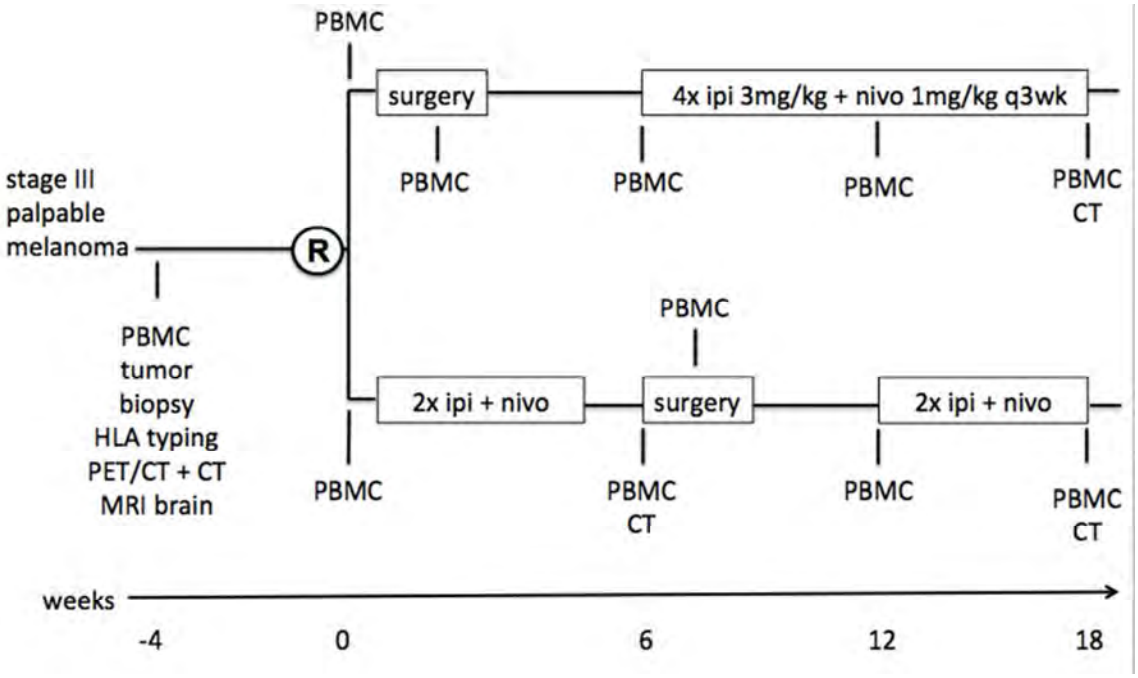

Figure 1: Schematic overview of OpACIN study**.**

4. STUDY POPULATION

4.1 Population

Resectable stage III melanoma patients with palpable lymph node metastases, no history or active in-transit metastases within the last 6 months, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, and being more than 18 years old will be eligible for this trial.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

• Adults at least 18 years of age

• World Health Organization (WHO) Performance Status 0 or 1

• Histologically confirmed resectable stage III melanoma with palpable lymph node metastases and no history or active in-transit metastases within the last 6 months

• Patient willing to undergo triple tumor biopsies during screening and in case of disease progression

• No prior immunotherapy targeting CTLA-4, PD-1 or PD-L1

• No immunosuppressive medications within 6 months prior study inclusion

• Presence of at least two of the defined HLA alleles (Table 1, see appendix)

• Screening laboratory values must meet the following criteria: WBC ≥ $2.0x10^9$/L, Neutrophils ≥$1.5x10^9$/L, Platelets ≥100 $x10^9$/L, Hemoglobin ≥5.5 mmol/L, Creatinine ≤1.5x ULN, AST ≤ 1.5 x ULN, ALT ≤ 1.5 x ULN, Bilirubin ≤1.5 X ULN

• normal LDH

• Women of childbearing potential (WOCBP) must use appropriate method(s) of contraception. WOCBP should use an adequate method to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug

• Women of childbearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of ipilimumab+nivolumab

• Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product

• Women who are not of childbearing potential (ie, who are postmenopausal or surgically sterile as well as azoospermic men do not require contraception

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

• Distantly metastasized melanoma

• Subjects with any active autoimmune disease or a documented history of autoimmune disease, or history of syndrome that required systemic steroids or immunosuppressive medications, except for subjects with vitiligo or resolved childhood asthma/atopy

• Prior CTLA-4 or PD-1/PD-L1 targeting immunotherapy

• Radiotherapy prior or post surgery within this trial

• Patients will be excluded if they are positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection

• Patients will be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS)

• Allergies and Adverse Drug Reaction

- History of allergy to study drug components
- History of severe hypersensitivity reaction to any monoclonal antibody

• Underlying medical conditions that, in the Investigator's opinion, will make the administration of study drug hazardous or obscure the interpretation of toxicity determination or adverse events;

• Concurrent medical condition requiring the use of immunosuppressive medications, or immunosuppressive doses of systemic or absorbable topical corticosteroids;

• Use of other investigational drugs before study drug administration 30 days and 5 half-times before study inclusion

• Pregnant or nursing.

4.4 Sample size calculation

The primary endpoint of the study is in particular the safety and feasibility of intermittent surgery during immunotherapy with nivolumab plus ipilimumab (neo-adjuvant arm). However, this needs to be contrasted to a therapy where the combination is given as adjuvant therapy (also experimental). The optimal way to gain experience with these approaches is by

randomizing in a phase 1b design. We therefore propose to randomize 20 patients to either receiving the combination of ipilimumab + nivolumab adjuvant, or to split neo-adjuvant and adjuvant with surgery in between (10 patients per arm). The study will be defined as not safe and feasible, if 2 out of the first 5 patients (point estimate 0.4 (95%CI 0.05-0.85)) or 4 out of the 10 (point estimate 0.4 (95%CI 0.12-0.74)) patients in the neo-adjuvant arm will experience immune-related adverse events leading to delayed surgery (not performed during week 6) or experience grade 3/4 SUSARs after surgery, that are attributed to the pre-surgery immunotherapy. The investigators realize that numbers of immune-related adverse events smaller than respectively 2 and 4 still bare a substantial chance of error of taking the wrong conclusion about safety, but they do not really expect adverse events.

The number of ten patients in each arm is chosen with the focus on producing relevant numbers of T cell responses that can be analyzed (immune-activating capacity). In patients with metastatic melanoma, neo-antigen specific T cell responses are observed in the majority of patients (>75%) by the technology used here (75% CD8+ T cell responses and 80% CD4+ T cell responses, T. Schumacher, unpublished data).

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The patients will be treated with nivolumab 1 mg/kg, 4 times, every 3 weeks, and with ipilimumab 3 mg/kg, 4 times, every 3 weeks.

Patients in Arm A will first undergo surgery and start 6 weeks later the combination of nivolumab+ipilimumab for12 weeks contiguous. Arm B will receive the combination for 6 weeks, undergo surgery and receive 6 weeks later the combination for another 6 weeks.

5.2 Use of co-intervention

Prohibited medication:

1) Concurrent chemotherapy, hormonal therapy, immunotherapy regimens, or radiation therapy, standard or investigational.

2) Use of growth factors including, but not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), or erythropoietin stimulating agents are not permitted, unless deemed necessary by investigator and discussed with medical monitor.

3) Use of systemic corticosteroids at > 10 mg daily prednisone equivalent, unless required for the treatment of infusion reactions, other adverse events, or for palliation as determined by the investigator.

4) Steroids must not be given as prophylactic anti-emetic therapy.

5) Use of herbal remedies is not permitted

Permitted medication:

The use of prescription and over-the-counter medications (except medications from categories outlined above) is permitted at the discretion of the Investigator and must be recorded on CRF.

Subjects are permitted the use of topical, ocular, intranasal, intra-articular, and inhalational corticosteroids (with minimal systemic absorption). Immunosuppressive doses (e.g., prednisone > 10 mg/day or equivalent) and/or physiologic replacement doses of systemic corticosteroids (e.g., prednisone <10 mg/day) are permitted in the context of treating adverse events. A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

Prophylactic anti-emetics, with the exception of steroids, may be administered at the discretion of the treating physician before any doses of study drug.

Use of the seasonal killed influenza vaccine during therapy is permitted without restriction. However, influenza vaccines containing live attenuated virus (Flumist ®) or other clinically indicated vaccinations for infectious diseases (killed or attenuated, e.g., Pneumovax ®, varicella, MMR, etc) may be permitted, but must be discussed with the PI and may require a study drug washout period prior to and after administration of the vaccine.

Contraception:

A Woman of Childbearing Potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, females under the age of 55 must have a serum follicle stimulating hormone, (FSH) level > 40mIU/mL to confirm menopause.

Females treated with hormone replacement therapy, (HRT) are likely to have artificially

suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is > 40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products, (rings, creams, gels)
- 4 week minimum for transdermal products
- 8 week minimum for oral products

Other parenteral products may require as long as 6 months.

5.3 Escape medication (if applicable)

All immune related adverse events should be treated according to standard algorithms (see appendix)

5.4 Safety and feasibility definition

The primary safety endpoint of the study is the safety and feasibility of intermittent surgery during immunotherapy with nivolumab plus ipilimumab (neo-adjuvant arm). The study will be defined as not to be safe and feasible, if 2 out of 5 patients or 4 out of the 10 patients in the

neo-adjuvant arm will experience immune-related adverse events leading to delayed surgery (not performed during week 6) or experience grade 3/4 SUSARs after surgery, that are attributed to the pre-surgery immunotherapy. In that case the principal investigator will discuss with the ethical committee and BMS the early termination of the study. During this review no patients will be included in the study.

For the adjuvant treatment arm are no new risks expected than the known toxicities that have been described in the investigators brochure. SUSARs will be reported as depicted in chapter 8.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Nivolumab 1 mg/kg, every 3 weeks, administered 4 times (12 weeks in total).

Ipilimumab (Yervoy) 3 mg/kg, every 3 weeks, administered 4 times (12 weeks in total).

Treatment schedule		
week	Arm A	Arm B
-4 till 0	Screening/BL	Screening/BL
0	**surgery**	nivolumab ipilimumab
1	x	x
2	x	x
3	x	nivolumab ipilimumab
4	x	x
5	x	x
6	nivolumab ipilimumab	**surgery**
7	x	x
8	x	x
9	nivolumab ipilimumab	x
10	x	x
11	x	x
12	nivolumab ipilimumab	nivolumab ipilimumab
13	x	x
14	x	x
15	nivolumab ipilimumab	nivolumab ipilimumab
16	x	x
17	x	x
18	x	x

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/ Label Type	Secondary Packaging (Qty) /Label Type	Appearance	Storage Conditions (per label)
Nivolumab/BMS-936558-01 Solution for Injection	100 mg (10mg/mL)	10 mL/open label	10 vials per box/ open labels	Colorless to pale yellow, clear to opalescent solution; may contain particles	Store Refrigerated, 2-8° C (36-46 F) Protect from light and freezing.
Ipilimumab Solution for Injection	200 mg (5 mg/mL)	40 mL per vial/Open label	4 vials per carton/Open label	Clear, colorless to pale yellow liquid. May contain particles	2 to 8°C. Protect from light and freezing.

6.2 Preclinical Summary of Nivolumab combined with Ipilimumab

Preclinical data indicate that the combination of PD-1 and CTLA-4 receptor blockade may improve antitumor activity. In vitro combinations of nivolumab plus ipilimumab increase IFN-production 2- to 7-fold over either agent alone in a mixed lymphocyte reaction. Increased antitumor activity of the combination was also observed in 3 of 5 syngeneic murine cancer models. In a murine melanoma vaccine model, blockade with either CTLA-4 or PD-1 antibodies increased the proportion of CTLA-4 and PD-1-expressing CD4/CD8 tumor infiltrating T effector cells, and dual blockade increased tumor infiltration of T effector cells and decreased intratumoral T regulatory cells, as compared to either agent alone [35].

Preclinically, a 4-week toxicity study of nivolumab in combination with ipilimumab conducted in cynomolgus monkeys demonstrated that the combination of nivolumab and ipilimumab resulted in dose-dependent gastrointestinal (GI) toxicity. Histologic findings included inflammatory changes in the large intestine, which increased in incidence and severity in a dose-dependent manner. GI toxicity/colitis was not observed in cynomolgus monkeys administered nivolumab alone, but was observed in monkeys receiving ipilimumab. Nivolumab in combination with ipilimumab was also associated with lymphoid hypocellularity of the cortex and/or medulla of the thymus and with acinar cell degranulation in the pancreas. Additional findings included interstitial mononuclear cell infiltrates in the kidneys, portal mononuclear cell infiltrates in the liver and myeloid hypercellularity in the bone marrow. Nivolumab in combination with ipilimumab at the high-dose level (ie, 50 mg/kg and 10 mg/kg, respectively) was associated with the death of 1 animal, attributed to acute gastric dilatation without histopathological evidence of colitis upon pathology evaluation of the GI tract.

6.3 Summary of findings from clinical studies

Ipilimumab Monotherapy

In MDX010-20, the ipilimumab monotherapy arm was administered 3 mg/kg ipilimumab every 3 weeks for four doses. In this arm, there were 79% drug related adverse events, with 21% being Grade 3/4 and 3/131 (2%) Grade 5. The most frequent adverse events of interest were rash (30%), pruritis (33%), diarrhea (33%), colitis (8%), endocrine disorders (9%), AST/ALT increased (2%), and hepatitis (1%). Any grade immune related adverse events were 60% and the Grade 3/4 immune related adverse events for the same cohort was 13% with the most frequent adverse events being diarrhea (5%), colitis (5%), rash (2%), and endocrine disorders (3%). Additional details on the safety profile of ipilimumab, including results from other clinical studies, are also available in the ipilimumab IB.

Nivolumab Monotherapy

One study has contributed most to the clinical experience with nivolumab monotherapy in subjects with melanoma and other solid malignancies. CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 304 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 03-Jul-2012, a total of 107 melanoma subjects were treated with nivolumab in the dose range of 0.1-10 mg/kg. No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 72.4% of subjects. The most frequent nivolumab related AEs occurring in ≥5% of subjects included: fatigue (25.7%), rash (13.5%), diarrhea (11.8%), pruritis (10.2%), nausea (7.9%), decreased appetite (7.9%), hemoglobin decreased (5.9%) and pyrexia (5.3%). The majority of events were low grade, with grade 3-4 drug related AEs observed in 14.8% of subjects. The most common Grade 3-4 drug-related AEs occurring in ≥ 1% of subjects were: fatigue (1.6%), lymphopenia (1.3%), abdominal pain (1%), diarrhea (1%), hypophosphatemia (1%) and pneumonitis (1%). At least one SAE was reported for 150 (49.3%) of the 304 subjects at all dose levels. Grade 3-4 SAEs were reported for 23 subjects (7.6%). Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%). Additional select treatment-related AEs have occurred with low frequency (< 5%) but are considered clinically meaningful, as they require greater vigilance for early recognition and prompt intervention. These AEs include: ALT increased (4.3%), AST

increased (3.6%), pneumonitis (3.3%), hypothyroidism (3.0%), hyperthyroidism (1.3%), renal failure (1.0%), adrenal insufficiency (0.7%) and colitis (0.7%). Grade 3-4 events of pneumonitis were reported in 3 subjects (1.0%) as described above (1 event was Grade 4). Grade 3 events of colitis, ALT increased, and AST increased were reported in 2 subjects (0.7%) each. Grade 3 events of adrenal insufficiency, hyperthyroidism, and hypothyroidism were reported in 1 subject (0.3%) each. Treatment-related AEs leading to discontinuation were reported in 18 (5.9%) of the 304 treated subjects on CA209003. The only events reported in more than 1 subject were pneumonitis (4 subjects; 1.3%) and hepatitis (2 subjects; 0.7%). There were 3 (1%) drug related deaths; each occurred after development of pneumonitis. Preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported.

The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in nivolumab exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with nivolumab during pregnancy. Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the nivolumab IB.

Nivolumab Combined with Ipilimumab.

In the Phase 1 study CA209004, ascending doses of nivolumab have been studied concomitantly with ascending doses of ipilimumab in subjects with unresectable or metastatic melanoma. In each arm in this multi-arm study, ipilimumab was administered once every 3 weeks for 4 doses with nivolumab administered once every 3 weeks for 8 doses. Starting at week 24, ipilimumab and nivolumab were administered once every 12 weeks for 8 doses. The three initial dose-escalation cohorts consisted of Cohort 1 (nivolumab 0.3 mg/kg plus ipilimumab 3 mg/kg; n=14), Cohort 2 (nivolumab 1.0 mg/kg plus ipilimumab 3 mg/kg; n=17) and Cohort 3 (nivolumab 3.0 mg/kg plus ipilimumab 3 mg/kg; n=6). Later, the study was amended to include Cohort 2a which evaluated nivolumab 3 mg/kg plus ipilimumab 1 mg/kg (n-16). The following DLTs were observed in Cohort 1 - Grade 3 elevated AST/ALT (1 subject); in Cohort 2 - Grade 3 uveitis (1 subject) and Grade 3 elevated AST/ALT (1 subject) and in Cohort 3 - Grade 4 elevated lipase (2 subjects) and Grade 3 elevated lipase (1 subject). Based on these data, Cohort 2 was identified as the maximum tolerated dose (MTD) and Cohort 3 exceeded the MTD. As of 15-Feb-2013, a total of 53 melanoma subjects were treated with nivolumab combined with ipilimumab in CA209004 across cohorts 1, 2, 2a, and 3. At least one AE regardless of causality has been reported in 98% of subjects treated. The most common (reported at > 10% incidence) treatment related AEs (any Grade %; Grade 3-4 %: 93; 53) are rash (55; 4), pruritus (47; 0), vitiligo (11; 0), fatigue (38; 0), pyrexia

(21, 0), diarrhea (34; 6), nausea (21, 0), vomiting (11, 2), ALT increased (21; 11), AST increased (21; 13), lipase increased (19; 13), amylase increased (15, 6), headache (11, 0), and cough (13, 0).

The majority of AEs leading to discontinuation (regardless of causality) were Grade 3 or 4 (reported in 11 of 53 subjects, 21%). Grade 3 events included lipase increased, ALT increased, AST increased, troponin I increased, colitis, diverticular perforation, pancreatitis, tachycardia, renal failure acute, choroiditis, autoimmune disorder, and pneumonitis. One subject each discontinued due to Grade 4 events of blood creatinine increased and AST increased. No drug-related deaths were reported.

6.4 Summary of known and potential risks and benefits

There is no standard systemic adjuvant immunotherapy for stage III melanoma. Participation in this trial sets the patients at high risk of developing immune related adverse events. Algorithms have been developed to treat patients developing irAEs. Recovery is commonly observed (except for endocrine irAE) and depends on the fast onset of the advised immunesuppression.

Local recurrences could be at a slightly higher rate due to neglecting local radiotherapy.

Systemic recurrences and overall survival could be improved by treatment with ipilimumab+nivolumab based on the promising data in stage IV melanoma patients, and early RFS benefit data from a phase 3 study of adjuvant ipilimumab after surgical treatment for stage III disease [1].

6.5 Description and justification of route of administration and dosage

Both ipilimumab and nivolumab are monoclonal antibodies and need to be i.v. infused. Dosing in this trial is identical to the recommended phase 2 dosage for ipilimumab+ nivolumab identified in stage IV melanoma patients [9]

6.6 Dosages, dosage modifications and method of administration

Nivolumab (1 mg/kg) is to be administered as an approximate 60 minute IV infusion, using a volumetric pump with a 0.2/0.22 micron in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline (per institutional standard of care). Details regarding the mixing and concentrations of the dose (preparation) and administration will be found in the current nivolumab IB.

Ipilimumab (3 mg/kg) is to be administered as an approximate 90-minute IV infusion, using a volumetric pump with a 0.2 to 1.2 micron in-line filter at the protocol-specified dose. Care must be taken to assure sterility of the prepared solutions, since the drug product does not contain any antimicrobial preservatives or bacteriostatic agents. Please refer to the ipilimumab IB for further details regarding preparation/administration.

Both study drugs are to be administered on the same day in this study. Nivolumab is to be always administered first. The nivolumab infusion must be promptly followed by a saline flush to clear the line of nivolumab before starting the ipilimumab infusion. The second infusion will always be the ipilimumab study drug, and will start no sooner than 30 minutes after completion of the nivolumab infusion.

6.7 Dose Delay Criteria

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories (see appendices).

Dose delay criteria apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab, ipilimumab or both). All study drugs must be delayed until treatment can resume.

Dose delay may not interfere with the timing of surgery in Arm B!

Nivolumab and ipilimumab administration should be delayed for the following:

- Any Grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related adverse event
- Any Grade 3 drug-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase, AST, ALT, or total bilirubin:
    - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay. It is recommended to consult with the principle investigator for Grade 3 amylase or lipase abnormalities.
    - If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity

- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

6.8 Criteria to Resume Treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (Section 6.9) should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the treatment will be stopped.

6.9 Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis,

bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions

- Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
- Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
  - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 8 x ULN
    - Total bilirubin > 5 x ULN
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN

- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab or ipilimumab dosing

6.10 Treatment of Nivolumab or Ipilimumab Related Infusion Reactions

Since nivolumab and ipilimumab contain only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE 4.0 guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [eg, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours).

Stop the nivolumab or ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab or ipilimumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab or ipilimumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [eg, renal impairment, pulmonary infiltrates]). Grade 4: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of nivolumab or ipilimumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with

methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab or ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (eg, appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (eg, oral antihistamine, or corticosteroids).

6.11 Preparation and labeling of Investigational Medicinal Product

Preparation and labeling of the investigational medicinal products will be performed by the pharmacy of the NKI according to in-house SOP.

6.12 Drug accountability

Drug accountability will be performed by the pharmacy of the NKI according to internal standards.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Primary readout will be the alteration in magnitude or breadth of the neo-antigen specific T cell response in the time interval pre- to post-adjuvant therapy in peripheral blood. To this purpose, the immunogenic mutational load of each patient's melanoma will be determined by DNA and RNA sequencing from baseline biopsies. Proteasomal degradation and peptide presentation in HLA will be predicted in silico. MHC-tetramer staining containing the predicted peptides will be done as described before (11).

In addition, we will analyze the effect of therapy on intratumoral T cell responses to obtain better insight into the mode of action of therapy. Identified neo-antigen specific T cells will be analyzed with respect to their phenotype and immunologic function (intracellular cytokine staining, lytic function as determined by CD107 staining, and coculture with APC presenting the cognate antigen).

Safety and feasibility as measured by SUSARs and adherence to the timelines in the study protocol.

7.1.2 Secondary study parameters/endpoints

RFS, will be determined according to RECIST 1.1 criteria

Rate and type of adverse events

Correlation between RFS and the delta of magnitude and/or breadth of neo-antigen T cell population

Differences between the two study arms concerning pharmacokinetics (serum antibody concentrations) as determined by enzyme-linked immunosorbent assay (ELISA), and pharmacodynamics as determined by PD-1 and CTLA-4 receptor occupancy on PBL

7.1.3 Other study parameters (if applicable)

n. a.

7.2 Randomization, blinding and treatment allocation

Patients fulfilling the eligibility criteria will be enrolled in the study. Patients will be randomized to receive either nivolumab+ipilimumab post-surgery (adjuvant, Arm A) or

intermitted surgery during nivolumab+ipilimumab (neo-adjuvant and adjuvant, Arm B). There is no blinding.

7.3 Study procedures

An overview of the study procedures can also be found in Table 2.

7.3.1 Screening phase

Following signing of the informed consent form for screening and enrolment into the study, the remainder of screening procedures and tests will be completed.

- Complete physical examination including height, weight, performance status, and vital signs, noting in detail the exact size and location of any lesions that exist.
- Tumor biopsies (3x 14g) for pathologic confirmation of stage 3b melanoma and genetic analysis of tumor. Patients may enter the study with a pathologic diagnosis of melanoma from any institution. Diagnosis must then be confirmed by the new biopsies at the NKI, Department of Pathology. If the diagnosis is not confirmed, the patient will be excluded from the study and replaced by another patient.
- Complete 4-digit HLA typing. Patients must have at least two of the HLA subtypes shown in Table 1. If the patient does not fulfill to this criteria, the patient will be excluded from the study and replaced by another patient.
- Hematology (Absolute neutrophil count (ANC), Hb, platelet count), Chemistry (ALAT/ ASAT, serum creatinine, total bilirubin))
- Baseline PET/CT and diagnostic CT of the neck, chest, abdomen and pelvis and brain MRI to evaluate the status of disease.
- ECG
- HIV antibody titer and HbsAg determination, Anti-HCV, anti-CMV antibody titer, and HSV serology, EBV panel and lues panel.
- beta-HCG pregnancy test on all women of child-bearing potential
- PBMC's and serum will be taken twice before start of treatment (once during screening and once 0-2 days before start of treatment)

7.3.2 During treatment

- The patients will be treated with 1 mg/kg nivolumab, 4 times, every 3 weeks, and with 3 mg/kg ipilimumab 4 times every 3 weeks.

Patients in Arm A will undergo surgery, after which they will start 6 weeks later with this combination for 12 weeks contiguous. Arm B will receive the combination for 6 weeks, undergo surgery (during 6 weeks interruption) and receive the combination another 6 weeks.

Tumor material (frozen and paraffin) will be collected for translational research from surgery material.

- Complete physical examination including height, weight, performance status, and vital signs week 0, 3, 6, 9, 12, 15, and 18.
- Hematology (Absolute neutrophil count (ANC), Hb, platelet count), Chemistry (ALAT/ ASAT, serum creatinine, total bilirubin)) week 0, 3, 6, 9, 12, 15, and 18
- CT of the neck, chest, abdomen and pelvis week 6 (neo-adjuvant arm only) and week 18 (both arms)
- From all patients PBMC's and serum will be taken during surgery (after tumor removal), week 6 (pre-surgery/pre-infusions), week 12 (pre-infusions), and week 18.

7.3.3 Post treatment evaluation

Patients will be evaluated every 3 months by physical examination and lab testing, up to three years. Subsequent structured follow-up will be according to the current melanoma guidelines (year 4 and 5 every 6 months, year 6 - 10 once a year).

In case of tumor relapse tumor biopsies (3x14G), PBMC and serum collection will be done.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

7.4.1 Specific criteria for withdrawal

If after having signed informed consent but before initiation of the treatment, patients do not fulfill the eligibility criteria anymore, patients will be withdrawn from the study.

7.5 Replacement of individual subjects after withdrawal

Patients that are withdrawn prior to initiation of the treatment and patients that do not fulfill the eligibility criteria will be replaced.

7.6 Follow-up of subjects withdrawn from treatment

Follow-up of patients withdrawn from the study will continue at least until 31 weeks after the treatment or until the treatment related toxicity has resolved to grade 2 or less (CTCAE 4.0). Thereafter, patients will receive standard follow-up for the disease.

7.7 Premature termination of the study

In case of unexpected toxicity or feasibility (see 5.4) the sponsor will discuss premature termination of the study with the ethical committee and with BMS.

Table 2. Schedule of assessments[1]	Screening/BL Week -4-0	Treatment Week 0	Week 3	Week 6	Week 9	Week 12	Week15	Week18	End of treatment	Follow-up[2]
Informed consent	x									
PA confirmation stage 3b melanoma	x									
HLA typing	x									
Medical history	x									
Physical examination*[3]	x	x	x	x	x	x	x	x	x	x
Hematology*[4]	x	x	x	x	x	x	x	x	x	x
Chemistry*[5]	x	x	x	x	x	x	x	x	x	x
Coagulation: PT/INR and aPTT	x									
beta-HCG pregnancy test[6]	x	x	x	x	x	x	x	x	x	
Serology[7]	x									
PET/CT	x									
Brain MRI*	x									
CT neck, chest, abdomen, pelvis	x			x[8]				x	x	x
ECG*	x									
Adverse events[9]		x	x	x	x	x	x	x	x	x
Blood for PBMCs, serum[10]	x	x[11]		x[11]		x		x		x
Tumor biopsy	x[12]									x[13]

* And if clinically indicated

1 For treatment schedule see section 6.1

2 Follow-up every 3 months up to 3 years

3 WHO PS, weight, temperature, pulse, blood pressure

4 Hb, ANC, and platelet count, incl. differentiation, Hct

5 LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, glucose, lipase, TSH, fT4, ACTH, cortisol, LH, FSH, testosteron/oestradiol, S100, CRP, ESR.

6 only WOCBP

7 HIV, HbsAG, HCV, anti-CMV, HSV, EBV and lues

8 only neo-adjuvant arm

9 AEs will be graded according to CTCAE 4.0, SAE reported up to 100 days post last infusion

10 100 ml heparinized blood +10 ml serum; during screening and at baseline within 2 days before start treatment, week 6, week 12, week 18, and at timepoint of relapse during FU

11 during surgery, post tumor removal 100 ml heparinized blood

12 Three biopsies taken with a 14g needle

13 Three biopsies taken with a 14g needle at timepoint of relapse

8. Safety reporting

8.1.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)

• Potential drug induced liver injury, renal failure, or pneumonitis are also considered an important medical event.

• Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

• Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

8.2.3 Reporting of SAEs

All Serious Adverse Events (SAE) occurring from registration until 100 days after the last protocol treatment/administration should be documented and reported (the study specific SAE form should be used) immediately to the NKI-AVL Safety Desk by fax: 0031 (0)20-5122679 between 09.00 and 17.00 hours Monday to Friday. If a fax is not possible due to technical problems, the Data Centre should be contacted by telephone: 0031 (0)20-5122668) between 09.00 and 17.00 hours Monday to Friday.

The Safety Officer will notify the study coordinator immediately of any serious adverse event (as defined above) experienced by a patient. The study coordinator will evaluate the SAE and will decide whether the event reported could be related to the protocol treatment and whether it is, both, unexpected and serious (SUSAR, see 8.2.4.).

The NKI-AVL Safety Desk will report the SAE's by fax to the subsidizing party BMS Company Global Pharmacovigilance & Epidemiology, Fax Number: 609-818-3804

Email: Worldwide.safety@bms.com SAEs will be followed up by the safety department of the AVL. Updates on the SAE will be sent to BMS similarly.

In addition, adverse events will be reported by the NKI-AVL Safety Desk to the METC and the CCMO. All SAE's will be reported once yearly, as described in the section 8.3. SAE's will not be reported through the web portal ToetsingOnline to the METC.

Serious adverse events occurring more than 100 days after the last study medication will NOT be reported unless the investigator feels that the study drug or a protocol procedure may have caused the event.

Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as 'serious', UNLESS at least one of the following exceptions are met:

• the admission is pre-planned (e.g. elective or scheduled surgery, documented in the

patient's file);

• hospitalization for technical (e.g. study drug administration) , practical or social reasons, in the absence of an adverse event.

Common toxicities observed for progressive disease and events secondary to progressive disease are generally excluded from reporting. However, in cases where the specificity or severity of an event is not consistent with the risk information, the event should be reported.

All SAE reports will be filed in the Investigator Study File.

In case the SAE is unexpected a SUSAR will be reported (see section 8.2.4).

8.2.4 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:

- Summary of Product Characteristics (SPC) for an authorised medicinal product;
- Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

– SUSARs that have arisen in the clinical trial that was assessed by the METC;

– SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs will to be reported till 100 days after discontinuation of the study drug.

8.5 Data Safety Monitoring Board (DSMB) / Safety Committee

No DSMB will be set-up.

9. STATISTICAL ANALYSIS

See chapter 4.4 Sample size calculation.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki, (Declaration of Helsinki, 59th WMA General Assembly, Seoul, October 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf).

The protocol must be approved by the CCMO and competent authority (CA).

10.2 Recruitment and consent

Informed consent (IC)

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements.

The IC procedure will conform to the ICH guidelines on Good Clinical Practice. This implies that "the written IC form will be signed and personally dated by the patient or by the patient's legally acceptable representative".

Recruitment

It is the responsibility of the investigator to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding her rights, the objective and procedures of the trial and the possible risks involved. Confidentiality-related information will be provided. The written patient information must be given to each patient. Patients will be given sufficient time for consideration. An independent physician will be available in accordance with the requirements of the national law. It is the responsibility of the investigator to obtain signed informed consent from every patient prior to the start of any

study related procedure. The written patient information is part of the documentation reviewed by the MEC mentioned. The patient information letter and informed consent form are attached as a separate document.

10.3 Benefits and risks assessment, group relatedness

See page 16

10.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The NKI-AVL (sponsor)(also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;

2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;

3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.5 Incentives (if applicable)

Not applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Subject identification

Following enrolment a patient sequential identification number or code will be allocated to the patients. This number will be used for identification of the patients and should be reported on all case record forms (CRFs). Data and patient material will be handled confidentially and if possible anonymously. When it is necessary to trace data or material to an individual subject, we will use a subject identification number list to link the data to the subject. The code is based on the sequence of enrolment combined with month-year of the patients' birth date. The key to the code will be safeguarded by the Principle Investigator. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP).

11.2 Randomization of the patients

Patient randomization will only be accepted from authorized investigators or through their authorized data manager or authorized staff member. A patient can be randomized only after verification of eligibility. During the randomization procedure eligibility criteria and items of patients' informed consent are checked. Randomization will be done by the Trial Office of the AVL after receiving the original signed IC and after verification of the eligibility criteria.

Patients will be randomized to be treated in Arm A or Arm B.

11.3 Storage of patient material

Patient material will be stored at the NKI-AVL. The tissue will be stored at the core facility molecular pathology and biobanking (CFMPB). The peripheral blood monocytes will be generated from peripheral blood by ficoll and stored in liquid nitrogen at the immunology division of the NKI-AVL. Material that is not used for current translational research will be stored for up to 15 years.

11.4 Data management

All data that are relevant for the study will be collected on CRFs developed by the data center of the AVL. The completed CRFs must be reviewed, signed and dated by the principal investigator or sub-investigator. The data will be collected and kept at the NKI-AVL.

11.5 Monitoring and Quality Assurance

Source data verification of the CRFs and check of the Investigator Study File documents will be performed by the clinical research monitor of the NKI-AVL, according to the procedures described in the Monitor Plan.

11.6 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the investigator.

11.7 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.8 End of study report

The investigator will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with

the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.9 Public disclosure and publication policy

Prior to initiation, the study will be submitted to the Dutch National Trial register, which is a recognized and accepted by the World Health Organization and International Committee of Medical Journal Editors (ICMJE) and to the NCI's PDQ® Cancer Clinical Trials Registry. All the results will officially be published.

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APPENDICES – Table 1

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A*02:11
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B*15:02
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B*35:01
B*40:01
B*55:02
B*57:01
B*57:03
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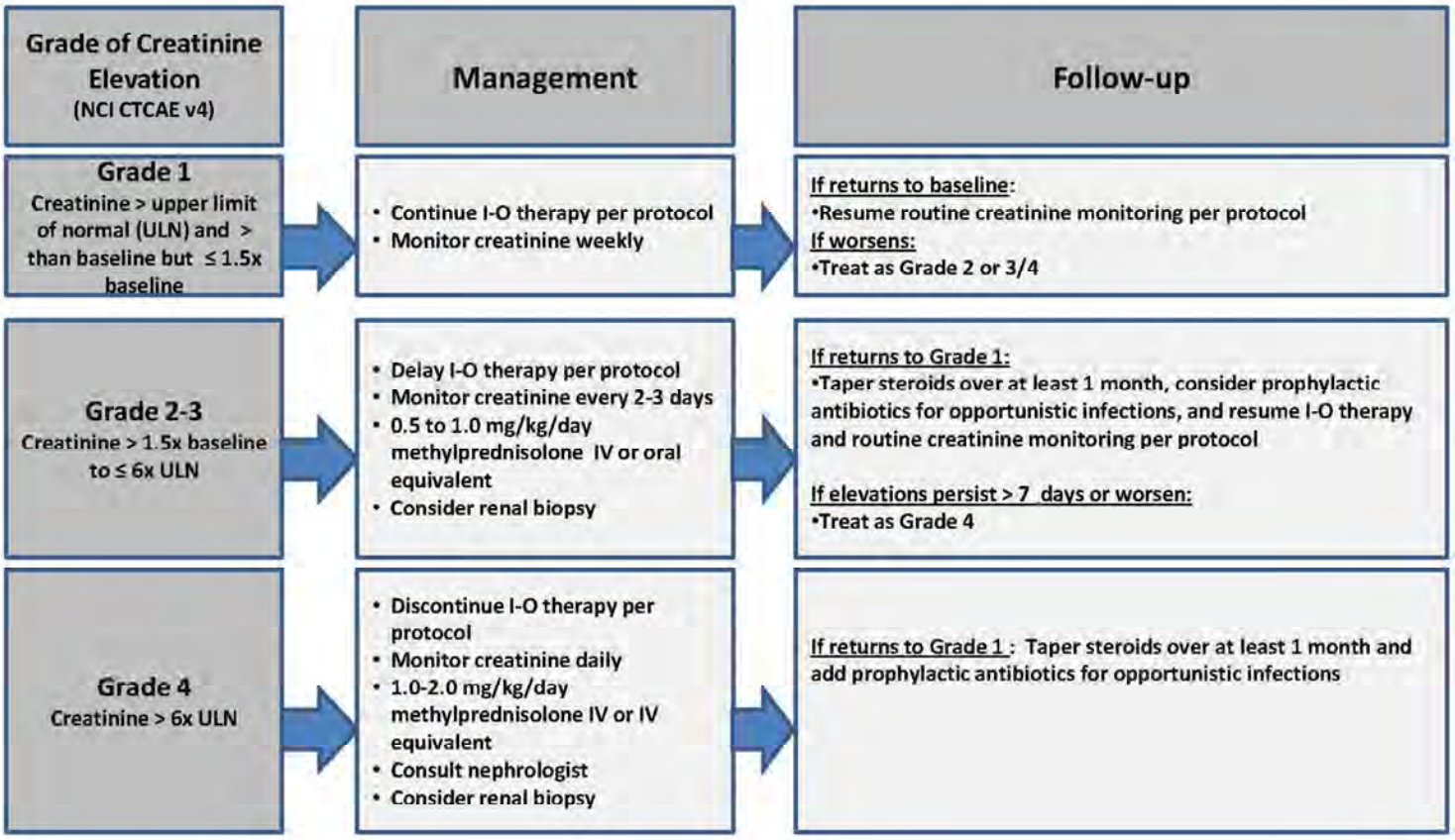
APPENDICES – SAFETY ALGORITHMS

Renal Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

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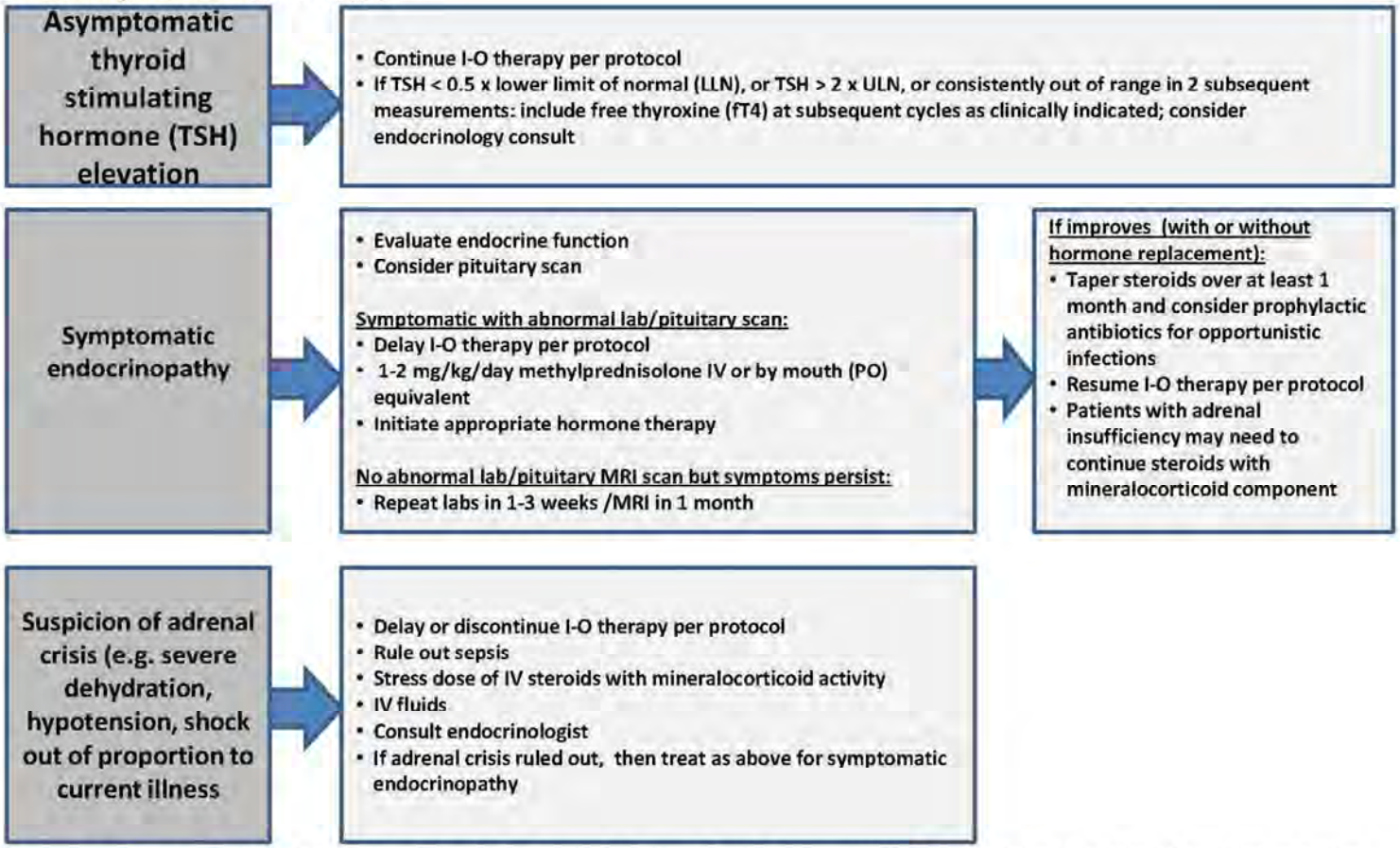
Endocrinopathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

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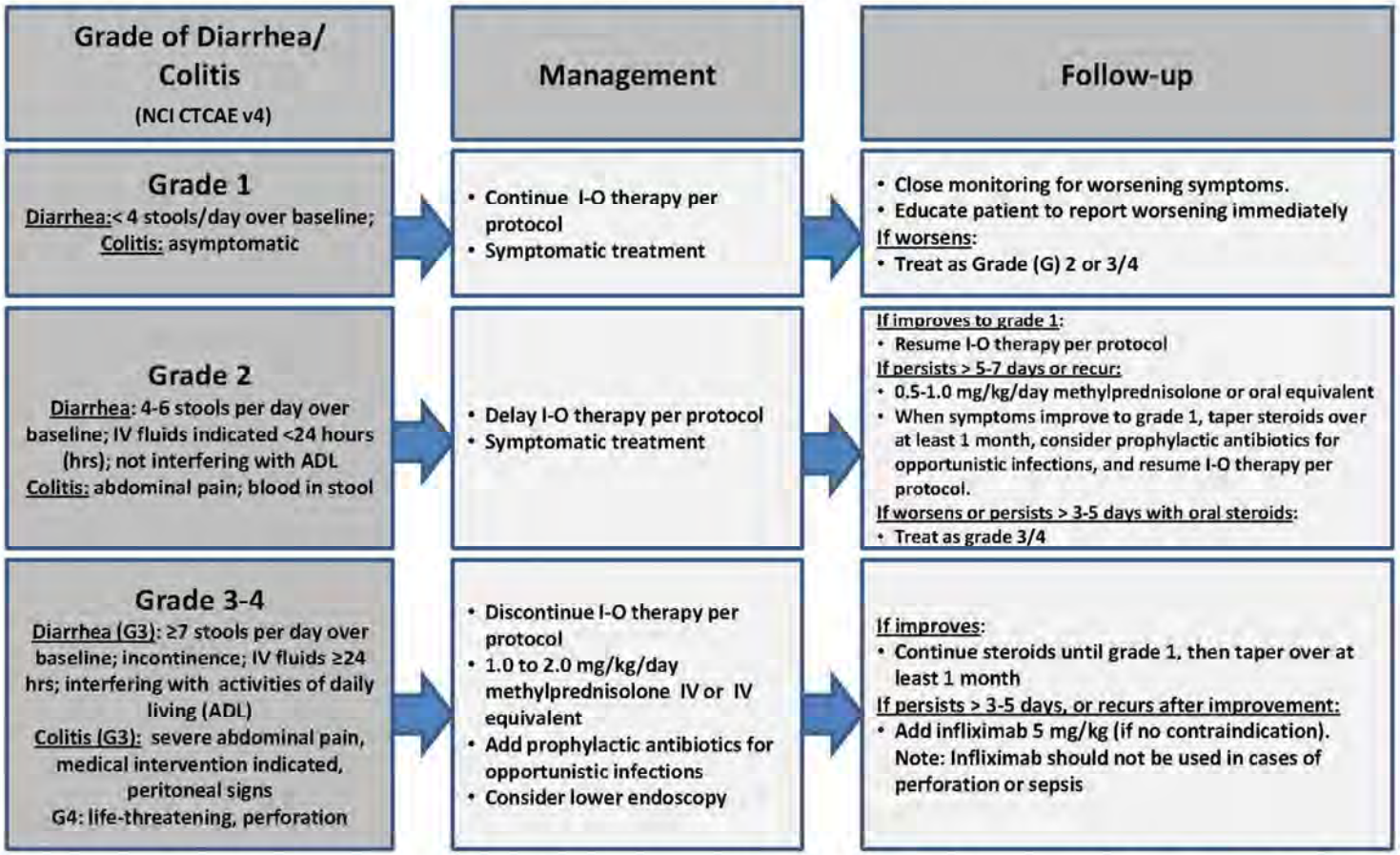
GI Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

Grade of Liver Test Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 AST or ALT > ULN to 3.0 x ULN and/or Total bilirubin (T. bili) > ULN - 1.5 x ULN	• Continue I-O therapy per protocol	• Continue liver function tests (LFT) monitoring per protocol If worsens: • Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x ULN and/or T. bili > 1.5 to ≤ 3 x ULN	• Delay I-O therapy per protocol • Increase frequency of monitoring to every 3 days	If returns to baseline: •Resume routine monitoring, resume I-O therapy per protocol If elevations persist > 5-7 days or worsen : • 0.5-1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol
Grade 3-4 AST or ALT > 5 x ULN and /or T.bili >3 x ULN	• Discontinue I-O therapy* • Increase frequency of monitoring to every 1-2 days • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent** • Add prophylactic antibiotics for opportunistic infections • Consult gastroenterologist	If returns to grade 2: • Taper steroids over at least 1 month If does not improve in >3-5 days, worsens or rebounds: • Add mycophenolate mofetil 1 gram (g) twice daily (BID) • If no response within an additional 3-5 days, consider other immunosuppressants per local guidelines

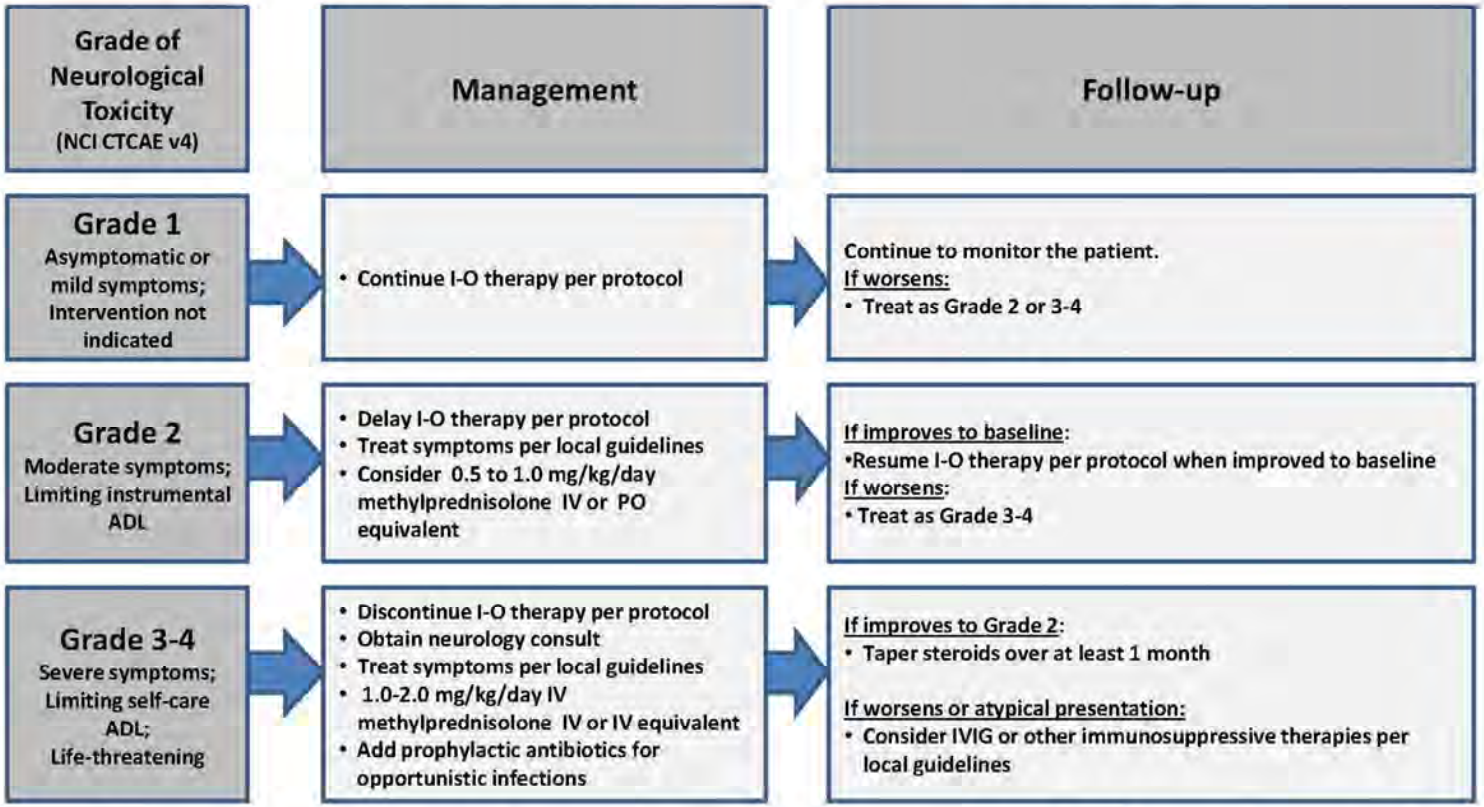
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Neurological Adverse Event Management Algorithm

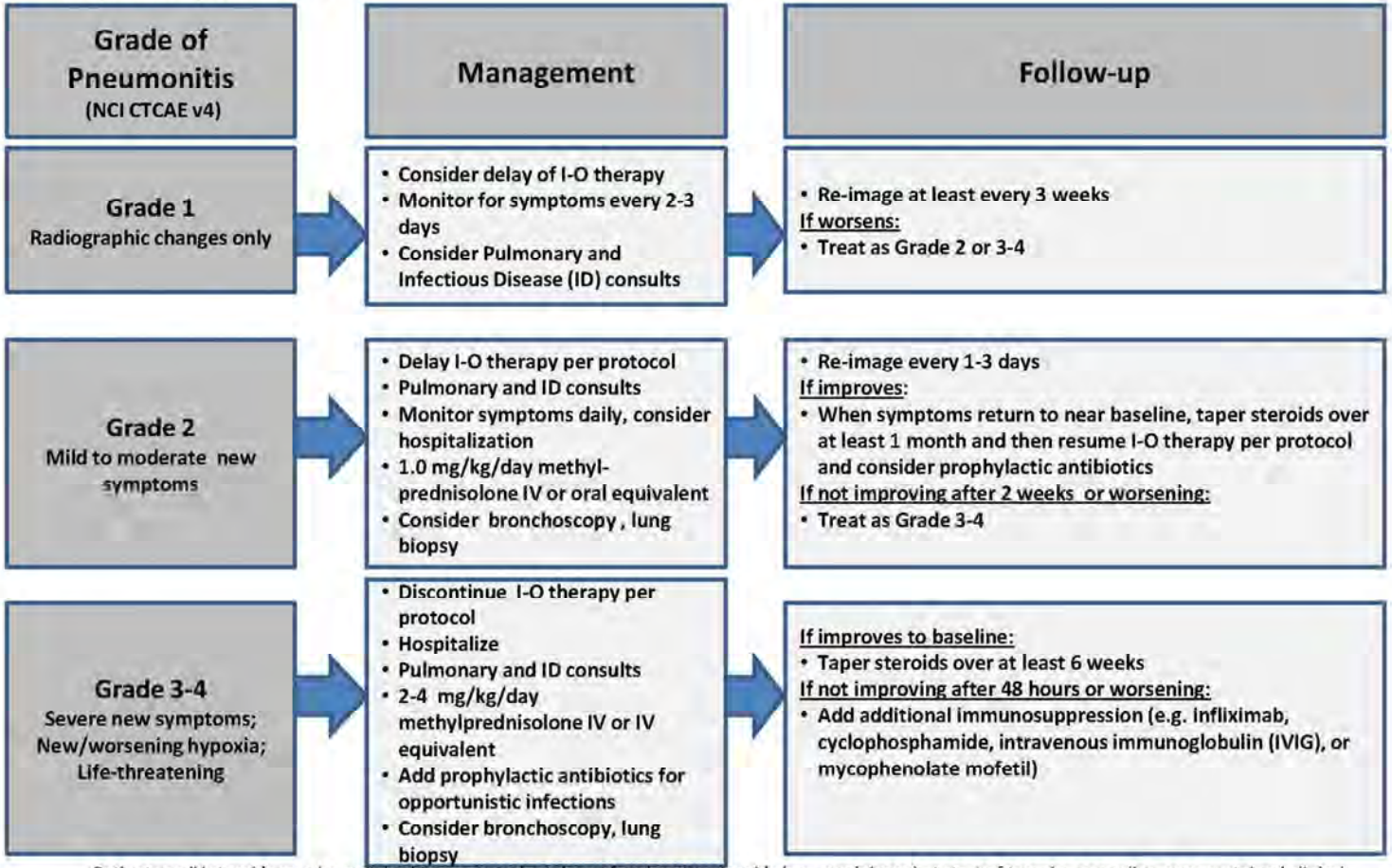
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

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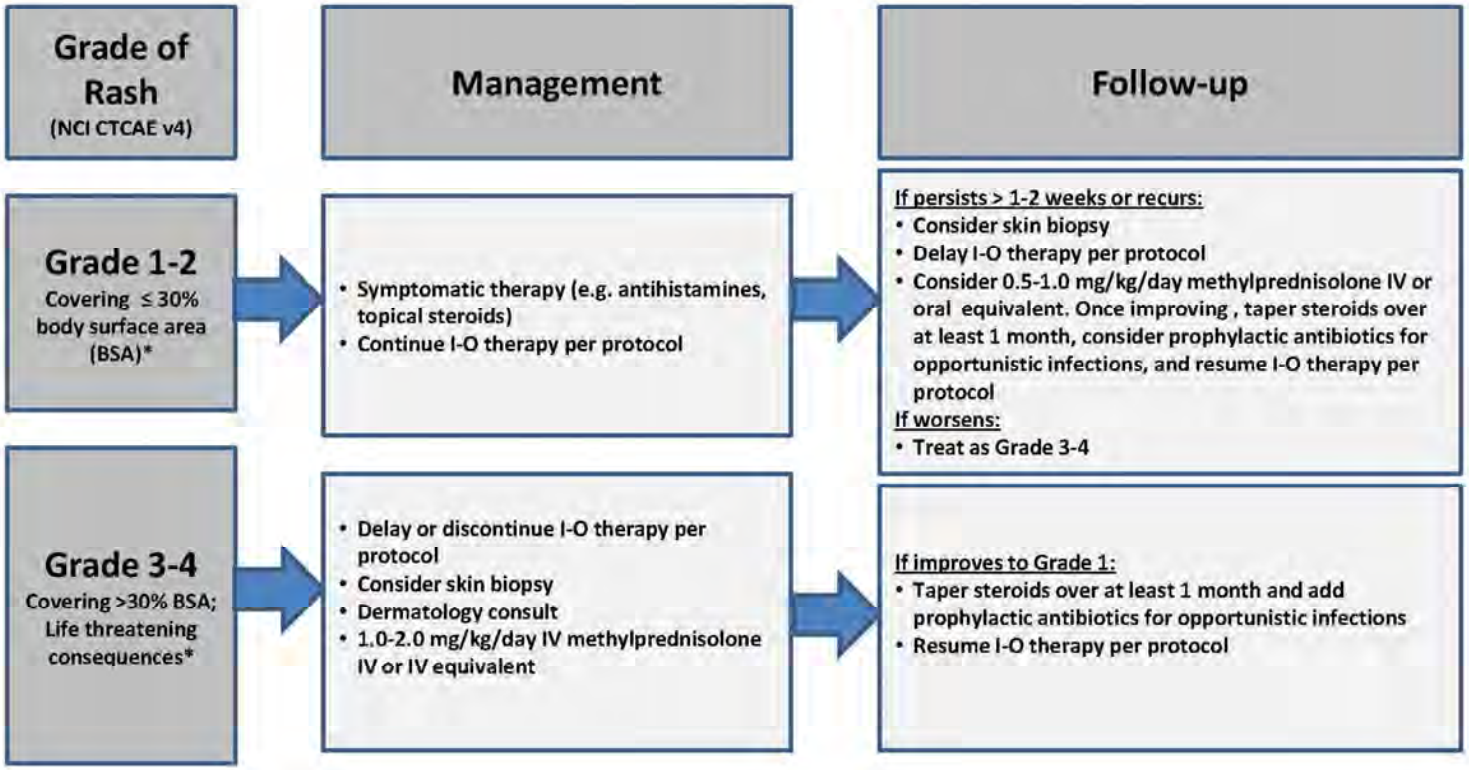
Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.