

## Explanation of literature-based nomenclature of the distinct signalling modules in MSI or MSS CRC *IFNG*<sup>+</sup>*CD8*<sup>+</sup> T cells

- **MSI CRC *IFNG*<sup>+</sup>*CD8*<sup>+</sup>T cells**

- **Stem-like progenitor:** This cluster had a distinct co-expression of *TCF7*, *IL2*, and *CD28*. It has been reported that stem-like precursor *CD8*<sup>+</sup> T cells highly express *TCF1* (coded by *TCF7*) together with some effector markers like *CD28*, further complemented by self-renewal capacity via pro-proliferation cytokines like *IL2* [1]. Hence, we termed this cluster as ‘stem-like progenitor’ signalling module.
- **Inflamed follicular-like:** This cluster had a distinct co-expression of *CXCR5*, *BCL6*, and *ICOS*. Herein, *BCL6* and *CXCR5* are well-established markers of follicular-like T cell phenotype wherein co-presence of *ICOS* marks a critical inflammatory signal to further facilitate *BCL6/CXCR5* associated follicular T cell differentiation [2]. Hence, we termed this cluster as ‘inflamed follicular-like’ signalling module.
- **Long-term immunosurveillance:** This cluster had a co-expression of *EOMES*, *TBX21*, *PRDM1*, *KLRK1*, and *KLRG1*. It has been reported that *EOMES*, *TBX21* and *PRDM1* together form the signalling ‘core’ of long-lived *CD8*<sup>+</sup> T cells that perform MHC-dependent immunosurveillance [3,4]. Similarly, *KLRK1* and *KLRG1* are well-established memory-like phenotype markers that also associate with temporally sustained immunosurveillance [3,4]. Given all the literature evidence, we named this group ‘long-term immunosurveillance’.
- **Tumour-reactive exhaustion:** This cluster had a co-expression of *IFNG*, *LAG3*, *PDCD1*, *CTLA4*, *TIGIT*, *ENTPD1*, *HAVCR2*, *CXCL13*, *NFATC2*. It has long been established that co-enrichment of effector genes like *IFNG*, *NFATC2*, *CXCL13*, together with immune-inhibitory receptors like *LAG3*, *PDCD1*, *CTLA4*, *TIGIT*, *ENTPD1* and *HAVCR2/TIM3* marks *CD8*<sup>+</sup> T cells that are tumour-reactive and show exhaustion on account of overexposure to tumoural antigens [5–9]. Hence, taken together, we termed this cluster as ‘tumour-reactive exhaustion’ signalling module.
- **Memory differentiation:** In this cluster, we identified co-expression of *TOX*, *IL15RA*, *NFATC3*, *NFIL3*, and *STAT1/2*. Genes like *TOX*, *NFATC3* and *NFIL3* mark memory differentiation in T cells such that effector programs linked to *IL15RA* and *STAT1/2* further potentiate or accompany pro-memory phenotypes [1,10]. Hence, ‘memory differentiation’ appeared to be the most accurate description for this cluster.

- **MSS CRC IFNG<sup>+</sup>CD8<sup>+</sup>T cells**

- **Long-term immunosurveillance:** This cluster had a co-expression of *EOMES*, *PRDM1*, *KLRK1*, *KLRG1*, *IL2*, and *TNF*. As mentioned above, at least *EOMES* and *PRDM1* are within the signalling ‘core’ of long-lived CD8<sup>+</sup>T cells performing MHC-dependent immunosurveillance [3,4]. Similarly, *KLRK1* and *KLRG1* co-mark memory-like phenotype also associated with temporally sustained immunosurveillance [3,4]. Finally, presence of *IL2* and *TNF* marks an effector T cell phenotype, which is functionally like the effector transcriptional programs sustained by *TBX21* in T cells [3,4]. As such, ‘long-term immunosurveillance’ appeared to be the most appropriate description of this signalling module.
- **Inflamed follicular-like:** This cluster had a distinct co-expression of *CXCR5*, *BCL6*, and *CD28*. As mentioned above, *BCL6* and *CXCR5* are well-established markers of follicular-like T cell phenotype [2]. Herein, *ICOS* and *CD28* are homologues of each other [2], so while unlike MSI CRC above, this cluster did not have *ICOS* co-expression yet presence of *CD28* can still mark increased inflammatory differentiation of follicular T cells [11]. Therefore, we termed this cluster as ‘inflamed follicular-like’.
- **Inflamed memory-like:** This cluster had a co-expression of *TOX*, *PDCD1*, *CTLA4*, *NFATC2/3*, *SMAD2/3/4*, and *STAT1/2*, *IL15RA*, *IL18RAP*. *TOX* and *NFATC2/3* mark memory differentiation in T cells such that effector programs linked to *IL15RA* and *STAT1/2* further potentiate or accompany pro-memory phenotypes [1,10]. Beyond this, this cluster also included a mixture of immune-inhibitory receptors (*PDCD1*, *CTLA4*), cytokine signalling (*IL18RAP*) and immuno-regulatory signalling (*SMAD2/3/4*) [12–14]. These ambiguously directed pathway together mark general inflammation, hence the label ‘inflamed memory-like’ for this signalling module.
- **IFN $\gamma$ -associated dysfunctional:** This cluster had co-expression of IFN- $\gamma$ -related genes like *TBX21*, *YY1*, *NFIL3*, *CXCL9*, *CXCL10* but negative correlations with other functional genes like *IL2*, *TNF*, *KLRK1*. Such contradictions are signs of dysfunction originating from incomplete or inappropriate activation of T cells, because correctly activated T cells tightly co-express activation programs associated with *IFNG*, *IL2* and *TNF* [15–18]. We therefore describe this cluster as ‘IFN $\gamma$ -associated dysfunctional’.
- **Tolerogenic c-Maf signalling:** This cluster had a co-expression of *MAF*, *ICOS*, *LAG3*, *TIGIT*, *ENTPD1*, and *HAVCR2*. It has been reported that *ICOS* is one of the inducers of *MAF* expression whereas *LAG3*, *TIGIT*, *ENTPD1* and *HAVCR2* are different immune-inhibitory receptor coding genes that are induced by the *MAF*-driven program [19]. Due to the omnipresence of upstream and downstream signalling centred around *MAF*, which tends to

have highly tolerogenic activity at colonic surfaces [19], we defined this cluster as the ‘tolerogenic c-Maf signalling’ signalling module.

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