Characterization of Tumor Infiltrating Dendritic Cells at the Single Cell Level

Marc Muller, Kyriaki Ioannou, Vaia Stavropoulou, Rupert Kenefeck, Anne Goubier and Ana Maria Florescu

Molecular Partners AG, Zurich-Schlieren, Switzerland

Introduction

CD40 is an attractive target for cancer immunotherapy due to its central role in dendritic cell activation and subsequent T cell priming against tumor antigens. Molecular Partners has developed a DARPin, MP0317, that activates CD40 in a FAP dependent manner, localizing the activation to the tumor microenvironment. Compared to systemic CD40 activation, this approach, which helps avoid the sink effect, might allow dosing that reduces toxicity due to systemic immune activation.

To support the translational understanding of MP0317's mode of action we used publicly available single cell sequencing data to develop a classifier for DC subpopulations, build an atlas of tumor infiltrating dendritic cells, and extract a signature specific to the CD40 expressing subpopulation. We plan to use this signature to evaluate changes in dendritic cell populations in clinical trials.

The DARPin Approach: Tumor Localized CD40 Agonism

DIRECT

1. Dendritic cells:
Enhanced antigen processing and presentation

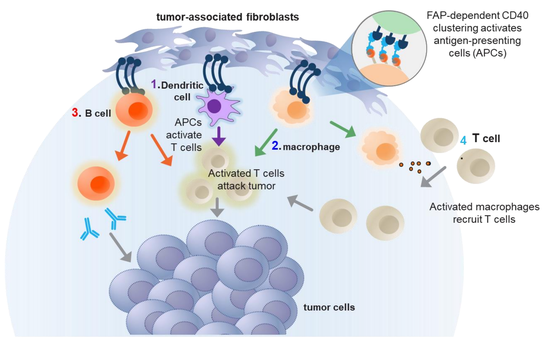
2. Macrophages:
Reporlarization of immune-suppressive M2 environment into pro-inflammatory M1 environment

3. B cells:
Activation of B-cells

INDIRECT

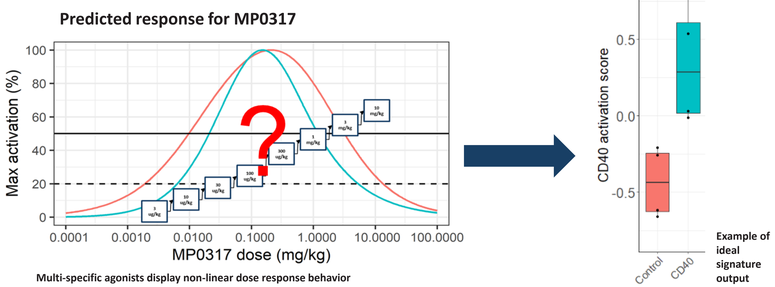
4. T-cells
Activation of T-cells to attack to tumor

MP0317, FAPxCD40, is expected to enhance therapeutic window via activating CD40 preferentially in FAP-expressing tumor microenvironment.



Tumor localized CD40 agonism. Our FAP-CD40 (MP0317) molecule DARPin activates antigen presenting cells in the tumor via FAP dependent clustering. CD40 agonism will activate antigen presenting cells (B cell , macrophages and dendritic cells) and, indirectly, T cells.

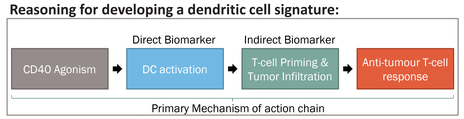
Gene Signatures Can Serve as Pharmacodynamics Biomarkers in Early-Stage Clinical Trials



Multi-specific agonists display non-linear dose response behavior

- For bispecific molecules the best dose is not always defined by the maximum tolerated dose
- Drug performance and optimal clinical phase 2 dosing must be informed by high quality biomarkers in early trials
- One of the biomarkers that can inform pharmacodynamics is the change in gene expression in tumor biopsies post treatment. However, in smaller patient cohorts changes in expression of individual genes can be hard to observe due to noise. As an alternative we can observe coordinated changes in genes relating to a biological mechanism or an immune cell type (gene signatures).

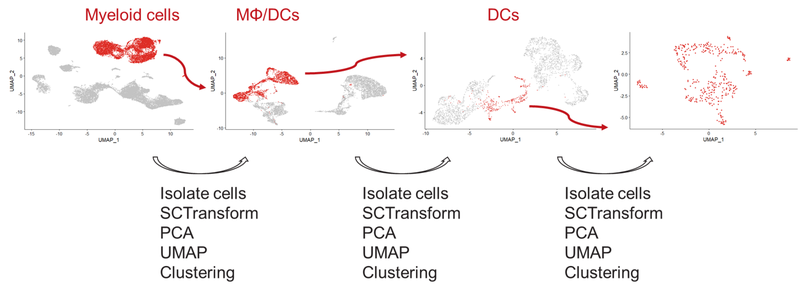
Reasoning for developing a dendritic cell signature:

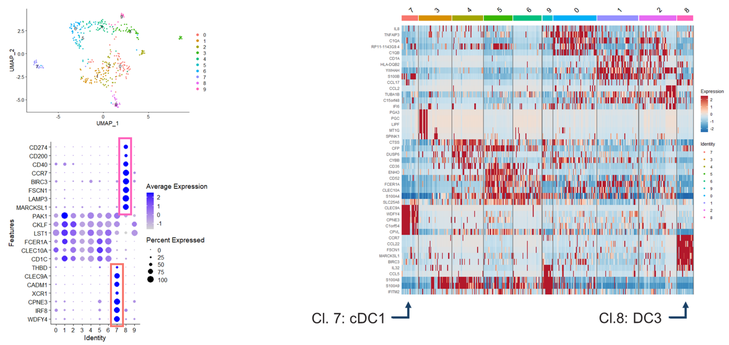


While T cell activation is a good biomarker we would also require a more direct one (DC activation). This will help show target engagement directly on antigen presenting cells.

Classifier for DCs: Training Set

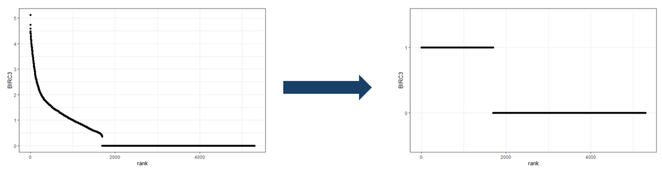
To obtain training data, multiple publicly available scRNA-Seq datasets were analyzed and annotated manually using the principle of iterative clustering:





Three populations (cDC1, DC3 and cDC2/moDC, as defined in reference 1) were identified: The DC1 group (cluster 7) corresponds to the conventional type I dendritic cells. We then observe a continuum of states across clusters 0 to 6 that starts with the conventional type II DCs and goes towards an intermediate macrophage/dendritic cell population. The DC3 in cluster 8 group corresponds to a mature state of DCs rather than a lineage and its presence has been recently shown in tumors [1,4].

Classifier for DCs: Algorithm



Example of rank expression and binarization for gene BIRC3

Algorithm steps:

1. Binarize data - to avoid batch effect bias when training the classifier, the expression data of the 4 datasets was converted to 0 or 1
2. Train random forest classifier on all available features
3. Select 200 features with highest discriminatory potential
4. Retrain classifier on those 200 features
5. Cells are first classified as cDC1 or DC3 using the classifiers and as moDC/cDC2 using the principle of exclusion

Summary of classifier performance:

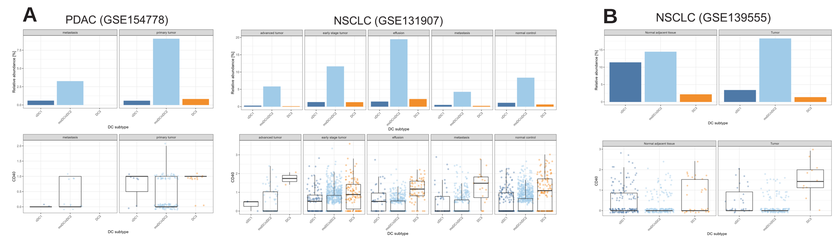
```
Confusion Matrix and Statistics

          Reference
Prediction    0    1
         0 6910   26
         1   13  494

               Accuracy : 0.9948
                 95% CI : (0.9928, 0.9963)
    No Information Rate : 0.9301
    P-Value [Acc > NIR] : < 2e-16

      Sensitivity : 0.9981
      Specificity : 0.9500
   Pos Pred Value : 0.9963
   Neg Pred Value : 0.9744
       Prevalence : 0.9301
   Detection Rate : 0.9284
```

DC Abundance and CD40 Expression Across Tumors:



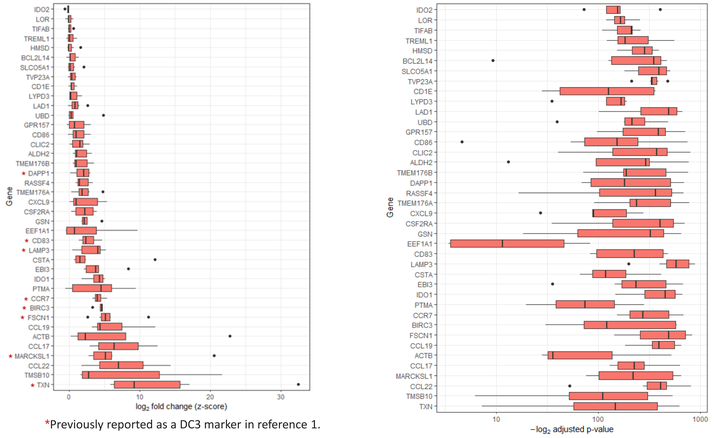
DC Abundance & CD40 expression across tumor stages:

A. Abundance (out of myeloid compartment) of DCs across tumor stages in PDAC and NSCLC. Abundance of both DC1 and 3 subtypes decreases in more advanced stages. DC3 group has the highest CD40 expression.

B. In early-stage tumors the DC abundance is higher than in normal tissues. CD40 expression is also higher in tumor infiltrating DC3s compared to normal tissue ones.

C. Immune checkpoint blockade increases abundance of DC1. Expression of CD40 is maintained on the DC3 compartment.

DC3 Signature for Use in Bulk RNAseq Analysis



*Previously reported as a DC3 marker in reference 1.

Using differential expression analysis (one versus all) and the most predictive features from our classifier we define a gene signature for the DC3 group. We are planning to use this signature in evaluating clinical biopsies together with previously established signatures for DC1 and DC2 populations [2,3].

Conclusions

- We have identified three types of tumor infiltrating DCs, in agreement with recent literature[1]
- We developed a classifier to call out these cells in multiple datasets originating from different tumor types.
- We quantified their abundance and CD40 expression across tissues and tumor stages and derived a gene signature that will be used for assessing pharmacodynamics effects in phase I clinical trials.
- This signature is currently being validated in *ex vivo* assays.
- This analysis also supports the hypothesis that earlier stage tumors would have a higher chance to respond to anti CD40 immunotherapy

1. Gerhard *et al.*, J. Exp. Med., 2020 Vol. 218 No. 1 e20200264 https://doi.org/10.1084/jem.20200264
2. Chen *et al.*, Met. Mol. Bio., 2018, 171810.1007/978-1-4939-7493-1_12
3. Becht *et al.*, Genome Biology, 2016, 17, 218
4. Maier et al, Nature 580, 257–262 (2020).

Molecular Partners AG, Wagistrasse 14, 8952 Zurich-Schlieren, Switzerland – www.molecularpartners.com – phone: +41 44 755 77 00