Sequencing and cloning IDH1 R132H targeted monoclonal T cell receptors from CD4+ T Cells facilitated by opto-electric-positioning technology

Duane Smith[2], Payal Watchmaker[1], Yelena Bronevetsky[2], Guido Stadler[2], Natalie Marks[2], Ryosuke Naka[1], Linda Ding, Xiaohua Wang, Hideho Okada[1,3,4], Kevin Chapman[2]

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Abstract

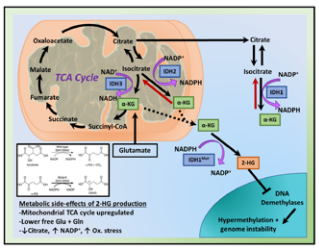
Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are enzymes that catalyze the conversion of isocitrate to α-ketoglutarate, while reducing NADH to NADPH. More than 80% of low-grade gliomas and secondary glioblastomas harbor point mutations in IDH. The R132H mutation is the most common mutation in IDH1 and vaccination with the mutant peptide elicited an target for MHC-class II-specific antitumor response, suggesting mutant IDH1 as a potential immunotherapy.(1) Discovering monoclonal T cell receptors (TCRs) that specifically recognize the R132H mutation, is a key, but challenging step towards viable immunotherapy for glioma/glioblastomas.

Using Opto-Electric-Positioning (OEP™) technology in a chip-based technology in a nanofluidic environment capable of culture, we have successfully identified and isolated a monoclonal T cell that specifically recognizes the R132H mutant IDH1 peptide.

We co-cultured monoclonal CD4⁺ T cells with stimulating monocyte derived dendritic cells that had been pulsed with R132H IDH1 peptide on chip. Activated, CD4⁺ T cells were selectively exported from the chip and their T cell receptors (TCRs) were subsequently deep sequenced and paired. The most likely pairs of alpha and beta TCR chains were cloned into mammalian expression vectors for functional validation in vitro, producing a TCR that specifically binds mutant IDH1 tetramer.

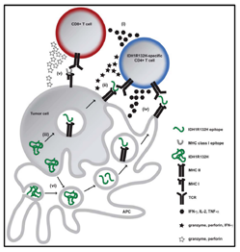
Taken together we show the combined platform of the OEP™ and nanofluidic chip provides a powerful solution to help identify cell type specific, monoclonal T cells that can be exported and sequenced to reveal distinct TCRs, which in turn can be cloned, expressed and validated for future immunotherapies.

Introduction



Mutant IDH1 alters metabolism and genome stability

IDH1^{Mut} catalyzes the conversion of α–ketoglutarate (α-KG) to 2-hydroxyglutarate (2-HG). 2-HG causes numerous metabolic imbalances and inhibits DNA demethylases.

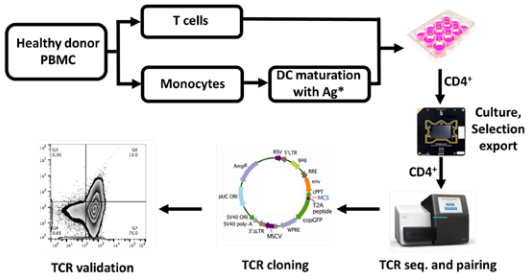


IDH1 Mutant antigen presentation

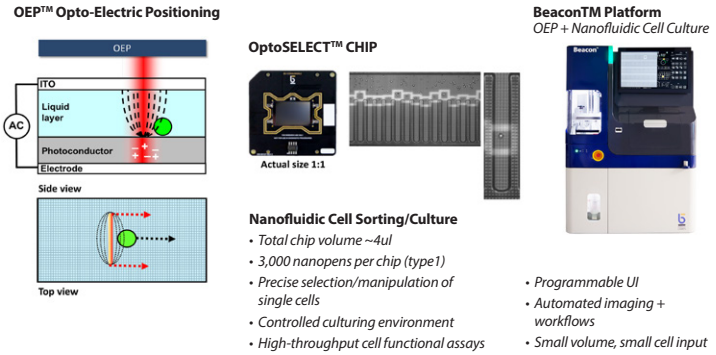
Mutant IDH1 antigens are presented by MHC class II complexes on antigen presenting cells (APCs). (2)

Synopsis

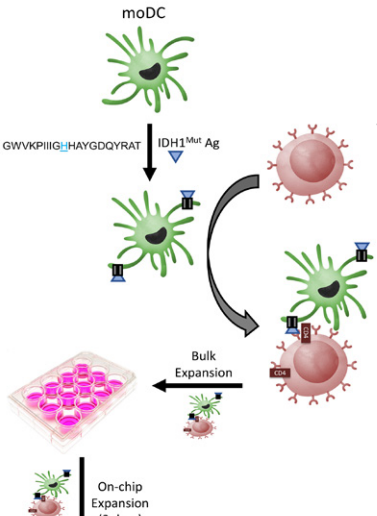
- Identify and expand clonal CD4⁺ T cells that recognize IDH1 R132H mutant antigen
- Sequence and pair TCR chains (alpha and beta)
- Clone paired TCRs into expression vector
- Validate expression and functionality of TCRs



T cell enrichment, expansion and selection

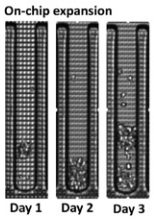


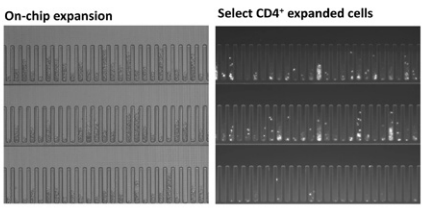
Using semiconductor manufacturing technology, an array of photo transistors is patterned on a silicon substrate. When activated by structured light, a local electric field is created, which can be used to move cells or other particles (e.g. beads) in fluid. Particles in a high dielectric medium (e.g. culturing media) will be repelled by the light structures. Wall structures can be built on the substrate to create NanoPenTM chambers, where cells can be placed. Once inside a NanoPen, the cell is protected from flow, as only diffusional transport occurs. By arraying NanoPens, a large number of cells can be isolated, analyzed and/or cultured in parallel.



IDH1 Mutant antigen pulsing

CD14⁺ monocytes were collected and matured into dendritic cells. Mature DCs were pulsed with IDH1 mutant peptide antigen (20mer) for MHC Class II presentation.
T cells were mixed with pulsed moDCs and expanded in bulk before enriching for CD4⁺ T cells. CD4⁺ T cells were imported on to a BLI Optoselect™ Chip.





Expansion of CD4+ T cells on OptoselectTM Chips

CD4⁺ T cells were co-penned with IDH1^{Mut} moDCs and cultured for 3 days on chip monitoring each pen for growth. After 3 days, ~5% of all pens expanded. Selected expanded pens were individually unpenned by OEP and exported into well plates for deep sequencing TCR chains.

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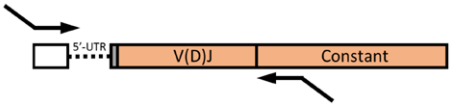
TCR sequencing and pairing



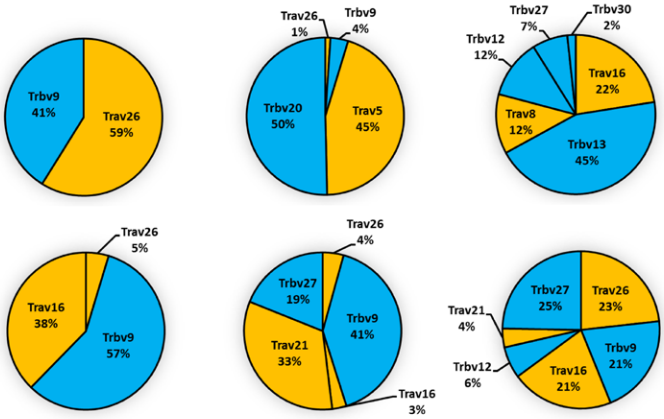
TCR Amplification

TCR V(D)J regions are amplified by directed PCR (α and β chains amplified separately then combined for indexing). Paired-end, long read sequencing reveals the V(D)J sequences of TCR chains.

Identifying TCR V(D)J and pairing α and β chains

Variable, diversity and junction (V(D)J) regions identified and relative abundance determined by MiXCR© *in silico.*

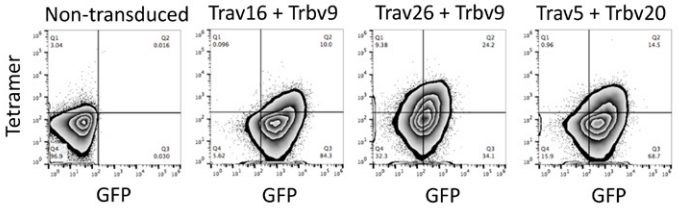
TCR chains are paired based on relative abundance vs. background samples and reoccurrence in other samples. Paired TCR sequences are cloned into expression vectors.



TCR cloning and validation

Cloning full length TCRs into expression vectors

TCR chains, including their natural signal peptides (grey boxes) were cloned into poly-cistronic expression vectors. Each ORF is separated by viral T2A peptide sequences to separate the chains during translation. GFP, downstream of the TCR chains was used as an expression marker.



IDH1 mut. Tetramer binding

Three TCR combinations have affinity for IDH1 mutant tetramer when transduced in Jurkat-76. *Additional validation experiments will be presented by Payal Watchmaker at Plenary Session 6 and poster A12.*

Conclusions

- CD4+ T cells were enriched and expanded from PBMC using moDCs presenting IDH1 R132H mutant peptide.
- Using growth on the nanofluidic chip as a phenotype, we down selected our candidate T cells, saving precious time and reagents in the downstream workflows.
- Deep sequencing TCRs from subset of expanded T cells revealed a number of potential TCR chain combinations.
- The most likely TCR chain combinations were cloned into expression vectors and validated for expression and functionality *in vitro.*
- Two TCR combinations show positive tetramer staining for IDH1 R132H mutant antigen.

References

1) Schumacher *et al,* **Nature,** 512, (2014): 324-327

2) Schumacher *et al,* **OncoImmunology,** 3:12 (2014):

3) Bolotin, *et al.* **Nature methods** 12, no. 5 (2015): 380-381.

4) Bolotin, *et al.*. **Nature Biotechnology** 35, 908–911 (2017)