Engineering tumor infiltrating lymphocytes from sarcoma and colorectal tumors with membrane bound IL15 for IL2 independent expansion and enhanced cytotoxicity against autologous tumor cell lines

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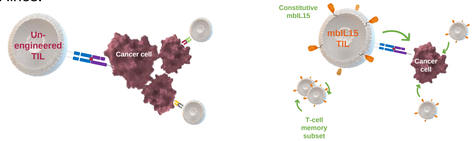
Abstract LB093

Background

Investigational tumor infiltrating lymphocytes (TIL) therapy has demonstrated encouraging efficacy in melanoma due to its advantages of having diverse TCR repertoire, the intrinsic tumor homing properties with potential to overcome the tumor microenvironment (TME).[1-3] Current TIL manufacturing Rapid Expansion Protocol (REP) requires high dose interleukin 2 (IL2), which yields terminally differentiated and potentially exhausted TIL, which may limit efficacy. Current treatment regimens also require high dose IL2 administration to support TIL survival, which limits their clinical applications due to IL2 related toxicity. Additionally, challenges have been reported to effectively generate and expand TIL from immune excluded “cold” tumor types such as sarcoma and colorectal (CRC).[4] Obsidian’s technology to engineer TIL with membrane bound IL15 (mbIL15) expands TIL without IL2 and supports memory CD8+ expansion while retaining T cell receptor (TCR) diversity and functionality. Here, we evaluated IL2 independent expansion of TIL from “cold” tumor types with constitutive mbIL15 engineering and the functionality of these investigational mbIL15 engineered TIL and their cytotoxicity against autologous tumor cell lines.



Polyclonal	✓	✓
Intrinsic tumor homing	✓	✓
Potential to overcome TME	✓	✓
Pre-clinical persistence	✓	+++
Potential IL2 side effects	+++	None

Methods

TIL from sarcoma and CRC tumors were isolated and expanded first through a proprietary pre-Rapid Expansion Protocol (pre-REP). The pre-REP TIL were then engineered with constitutive mbIL15 expression by transduction with viral vectors and expanded with Obsidian's proprietary process *in vitro* with an engineered feeder cell line (iFeeder cells).[5] Autologous tumor cell lines were established *in vitro* with tumor type specific protocols and examined routinely for tumor cell purity. mbIL15 TIL were immunophenotyped and assessed for in vitro polyfunctionality as well as cytotoxicity against autologous cell lines. mbIL15 engineered TIL were compared with IL2 expanded, un-engineered TIL.

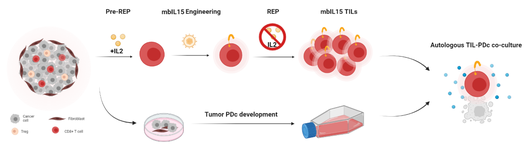


Figure 1. Schema mbIL15 TIL and autologous patient derived cell lines (PDc) generation. Tumors were processed into fragments put into pre-REP process. The pre-REP TIL were then engineered with mbIL15 by viral transduction. The transduced mbIL15 TIL were expanded using Obsidian’s proprietary REP process with engineered feeder cells (iFeeder cells). Dissociated tumor cells from the same donors were put into cell line development using indication specific protocol to generate autologous PDc. The autologous REP TIL and PDc were put into co-culture to study cytotoxicity (caspase readout).

Pre-REP from CRC and Sarcoma tumors yield high percentage of CD8 T cells

Obsidian’s robust process generates pre-REP TIL in all cases

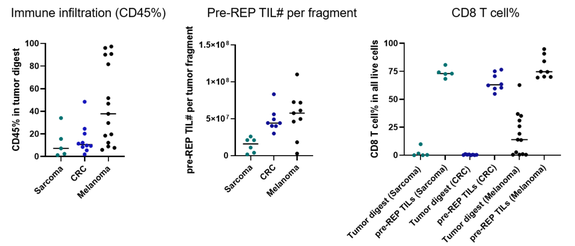


Figure 2. Obsidian’s process robustly generates pre-REP TIL from sarcoma and CRC tumors and enriches for CD8 T cells. (*Left*): Sarcoma and CRC tumors have less immune infiltration when compared with melanoma; (*Middle*): With Obsidian’s pre-REP process pre-REP TIL could be reliably expanded from sarcoma and CRC tumors despite the challenging low immune infiltration; (*Right*): Obsidian’s pre-REP process could expand and enrich CD8+ T cells from tumor samples.

Pre-REP TIL from sarcoma tumors show similar proliferative, functional phenotypes as melanoma

Figure 3 Single cell profiling of pre-REP TIL shows similar proliferative, functional CD8 T cell phenotypes across sarcoma and melanoma donors. For single-cell transcriptomic profiling, pre-REP TIL from sarcoma show similar expression profiles when compared with melanoma, including enrichment for CD8 (CD8A), proliferation marker Ki67 (MKI67), and expression of functional molecules including granzyme B (GZMB) and Granulysin (GNLY). Our comparative analysis of single-cell sequencing data showed that there were no differentially expressed genes (> 2-fold change with FDR < 0.05) across the sarcoma and melanoma pre-REP T cells; similarly, unbiased clustering showed that none of the 10 T cell clusters was restricted to only one tumor type.

mbIL15 engineering expand TIL and maintain memory phenotype in REP

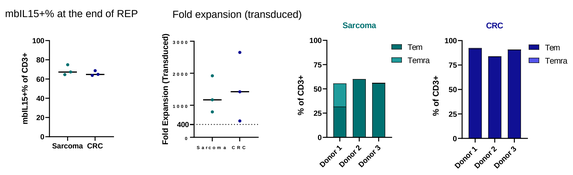
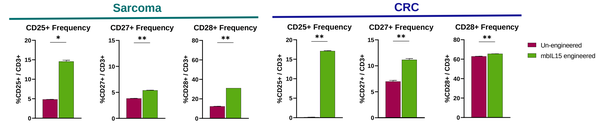


Figure 4. mbIL15 TIL demonstrate IL2 independent expansion and effector memory phenotype in REP. (*Left*): REP TIL from both Sarcoma and CRC TIL show high mbIL15+% and expansion independent of IL-2; (*Right*) Both sarcoma and CRC REP TIL show high percentage of effector memory (CD45RA-CD45RO+CCR7-CD62L-) phenotype.

mbIL15 engineered REP TIL show higher activation and lower exhaustion marker expression

Higher percentage of mbIL15 engineered TIL express activation markers



Lower percentage of mbIL15 engineered TIL express exhaustion markers

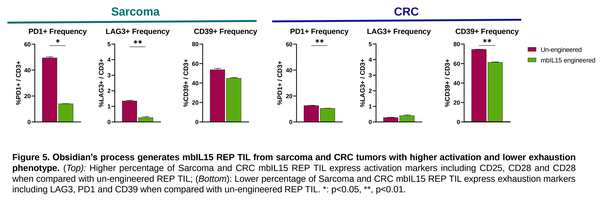


Figure 5. Obsidian’s process generates mbIL15 REP TIL from sarcoma and CRC tumors with higher activation and lower exhaustion phenotype. (*Top*): Higher percentage of Sarcoma and CRC mbIL15 REP TIL express activation markers including CD25, CD28 and CD28 when compared with un-engineered REP TIL; (*Bottom*): Lower percentage of Sarcoma and CRC mbIL15 REP TIL express exhaustion markers including LAG3, PD1 and CD39 when compared with un-engineered REP TIL. *: p<0.05, **, p<0.01.

mbIL15 engineered REP TIL retain TCR Vβ diversity

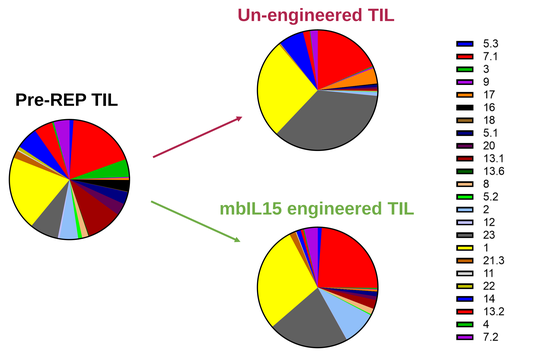


Figure 6. mbIL15 TIL retain diversity of TCR Vbeta chains. Pre-REP and REP TIL from sarcoma donor were profiled using Beta Mark TCR Vbeta repertoire kit (Beckman Coulter). REP TIL from sarcoma donor retained the diversity of TCR Vbeta chain.

mbIL15 engineered REP TIL show higher polyfunctionality than un-engineered TIL

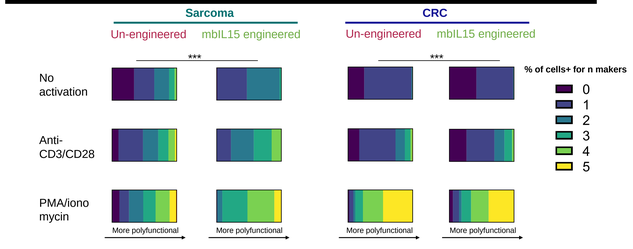


Figure 7. mbIL15 TIL show high polyfunctionality. Unengineered or mbIL15 engineered post-REP TIL were rested for 24 hours in IL-2 supported (for unengineered TIL) or cytokine-free media (for mbIL15 engineered TIL). The TIL were then activated overnight with anti-human CD3/CD28 followed by 6 hours transport inhibitor treatment or 6 hours of PMA/ionomycin treatment in the presence of transport inhibitors in IL-2 supported (for unengineered TIL) or cytokine-free media (for mbIL15 engineered TIL). The TIL were then analyzed by flow cytometry for makers of CD107a/perforin/interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and granzyme b. Percentage of cells that are positive for 0, 1 or more markers are represented above. ***Chi-square p<0.001.

mbIL15 engineered REP TIL exhibit enhanced cytotoxicity & IFN-γ secretion against autologous tumor cells

Figure 8. mbIL15 TIL show enhanced cytotoxicity and IFN-γ cytokine secretion against autologous tumor PDc when compared with unengineered TIL. (*Top*) REP TIL were co-cultured with autologous tumor cell line (PDc) and imaged using Incuyte imaging platform. Caspase signal was detected and analyzed in tumor-PDc co-culture group, TIL only group and PDc only group. Net Caspase+ was calculated by deducting the caspase 3/7 signal of the TIL only and PDc only group from the co-culture group; (*Bottom*) Supernatants from 24 hr TIL:PDc co-culture were collected and assayed for IFN-γ secretion. *: p<0.05, **: p<0.01, ***: p<0.001.

Conclusions

These data demonstrate:

- Obsidian has developed a robust, universal process to generate TIL from immunologically “cold” tumor types such as sarcoma and CRC, that are typically associated with limited T cell infiltration;
- Obsidian’s TIL process with mbIL15 engineering have highly enriched effector memory phenotype CD8 T cells in sarcoma and CRC and is comparable to our previous experience in melanoma;
- Consistent with our previous experience in melanoma , Obsidian’s mbIL15 engineered TIL demonstrate high TCR Vbeta diversity, exhibit higher activation status, polyfunctionality, and enhanced cytotoxicity and reactivity against autologous tumor cell lines when compared with unengineered, IL-2 expanded TIL in sarcoma and CRC.

References

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