361 UNIVERSAL EXPANSION OF CBL-B-INHIBITED TUMOR INFILTRATING LYMPHOCYTES, DETIL-0255, FROM WOMEN WITH OVARIAN CANCER: PROCESS VALIDATION

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Background Despite objective responses to immune checkpoint blockade in patients with ovarian cancer (OC), therapies providing durable clinical benefit are lacking.[1] An increased density of OC tumor infiltrating lymphocytes (TIL), specifically memory T cells with enhanced CD28 signaling, are associated with improved survival[2] and immunotherapy response.[3]Adoptive cell therapy (ACT) utilizing *ex vivo* expanded TIL has demonstrated durable complete responses in several epithelial malignancies, but has shown limited clinical benefit in OC.[4,5] This is due in part to extended manufacturing times and use of TIL products with a differentiated and exhausted phenotype.[4,5] Casitas B lineage lymphoma-B (CBL-B) is an E3 ubiquitin ligase that limits T cell activation in the absence of CD28 co-stimulation following T cell receptor engagement. *Ex vivo* inhibition of CBL-B with the small molecule inhibitor NX-0255 increases the expansion of stem-like TIL with enhanced *in vivo* tumor cytotoxicity and persistence compared to conventional TIL expanded in IL-2 alone.[6,7] Here we present our pre-clinical and early manufacturing experience of drug enhanced TIL therapy (DeTIL-0255) in OC.

Methods Tumor tissue from N=21 consenting patients undergoing resection for OC across multiple US clinical sites was fragmented and cultured with IL-2 and NX-0255 under research (N=20) or clinical scale (N=1) manufacturing conditions. Following 22 days of culture, DeTIL-0255 cell products were characterized by multiparameter spectral flow cytometry.

Results Even with as low as five input 2x2 mm^3 tumor fragments, DeTIL-0255 was reproducibly expanded from primary and metastatic OC lesions with an average fold increase of 184±179 (mean ± SD) following the rapid expansion protocol and Day 22 total viable cell count of $8.0x10^8 \pm 5.3x10^8$ cells (research scale) and $2.5x10^{10}$ cells (clinical scale). OC DeTIL-0255 was comprised of T (81.4±10.1%) and NKT (10.5±10.4%) cells with < 4% monocytes, NK, or B cells. OC DeTIL-0255 showed a balanced mixture of CD4 (53.4 ±28.9%) and CD8 (38.8±27.8%) T cells with heightened expression of the co-stimulatory marker CD226 (CD4: 87.4 ±12.6%; CD8: 86.0±16.4%), previously shown to predict immunotherapy response [8]. In contrast to prior OC TIL products, OC DeTIL-0255 were primarily effector memory (CD4: 59.7±30.6%; CD8: 55.6±29.8%) and central memory cells (CD4: 21.0±23.7%; CD8: 12.4±16.9%) displaying limited T cell exhaustion (CD4: PD-1 26.2±22.8%, LAG-3 15.1 ±13.5%, CD57 4.4±4.3%; CD8: PD-1 17.7±19.6%, LAG-3 45.4±28.4%, CD57 2.9±2.5%).

Conclusions OC DeTIL-0255 demonstrate a favorable phenotype amenable for ACT. A Phase 1 clinical study of DeTIL-0255 in women with recurrent/platinum resistant OC is ongoing (NCT05107739).

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Ethics Approval All studies were performed in full accordance with the guidelines for good clinical practice and the Declaration of Helsinki and approved by the cited institutional protocol review committee and IRB.

Consent Written informed consent was obtained from the patient for use of patient specimens for research and subsequent publication.

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