Costimulatory Antigen Receptor (CoStAR): A Novel Platform That Enhances the Activity of Tumor-Infiltrating Lymphocytes

Poster **198**

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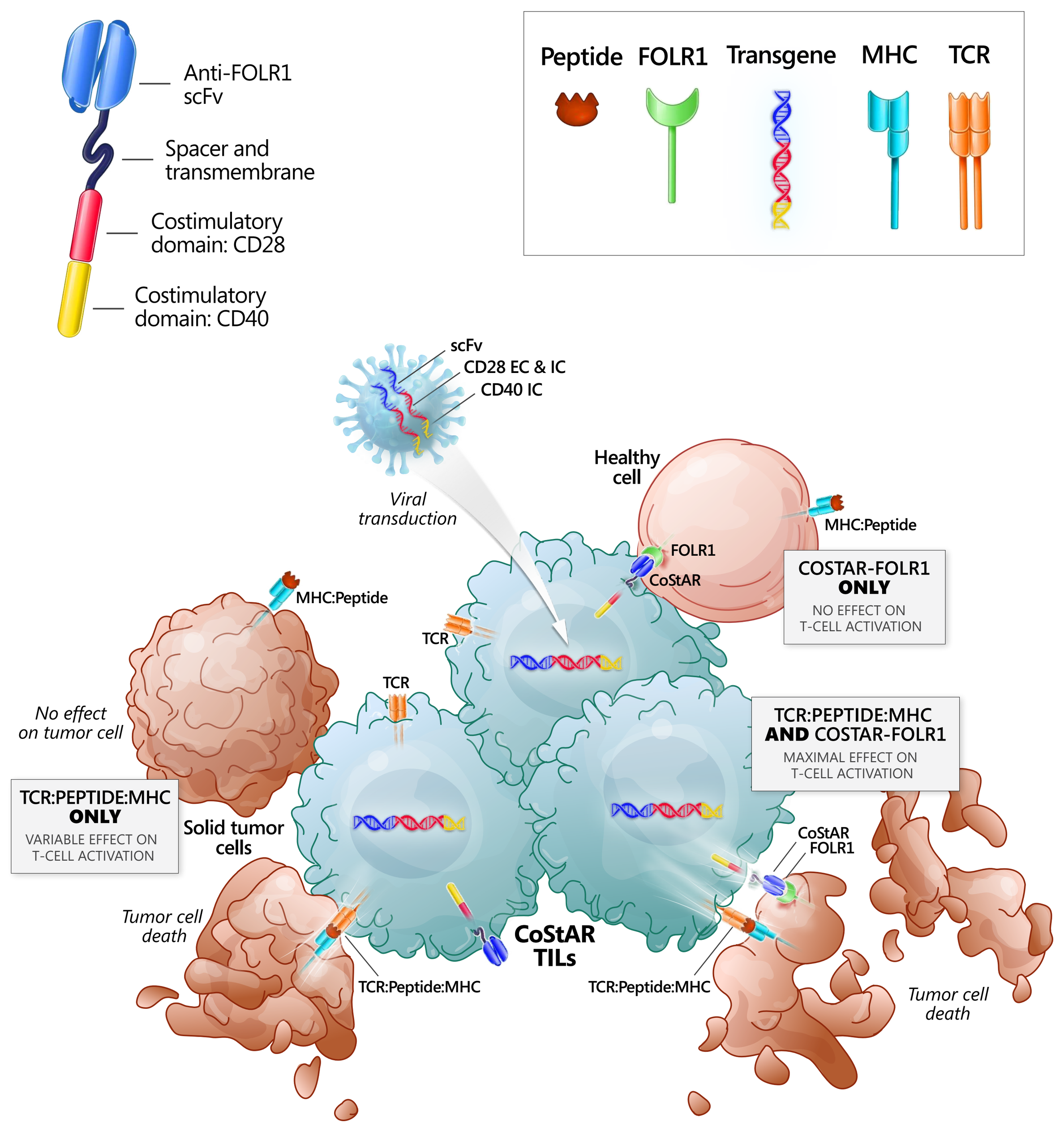
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BACKGROUND

- Major limiting factors for development of cell therapy modalities in solid tumors include clonal heterogeneity and the related lack of universally expressed tumor-specific antigens[1-3]
- As the only truly polyclonal cell product in advanced development, tumor-infiltrating lymphocytes (TILs) encompass the maximum possible diversity available with an unrestricted T-cell receptor repertoire (TCR), thereby offering the broadest diversity of anti-tumor reactivity[4,5]
- The suppressive tumor microenvironment is characterized by high expression of coinhibitory receptors and poor costimulation[6,7]
- To overcome poor endogenous costimulation, a novel platform was engineered to leverage the diverse TCR repertoire of TILs while amplifying their antitumor activity via a synthetic costimulatory antigen receptor (CoStAR; **Figure 1**)

Figure 1. CoStAR Platform Overview


Anti-FOLR1 scFv
Spacer and transmembrane
Costimulatory domain: CD28
Costimulatory domain: CD40
Peptide FOLR1 Transgene MHC TCR
scFv
CD28 EC & IC
CD40 IC
Viral transduction
Healthy cell
MHC:Peptide
FOLR1
CoStAR
COSTAR-FOLR1 ONLY
NO EFFECT ON T-CELL ACTIVATION
TCR
No effect on tumor cell
TCR:PEPTIDE:MHC ONLY
VARIABLE EFFECT ON T-CELL ACTIVATION
Solid tumor cells
TCR:PEPTIDE:MHC AND COSTAR-FOLR1
MAXIMAL EFFECT ON T-CELL ACTIVATION
Tumor cell death
CoStAR TILs
TCR:Peptide:MHC

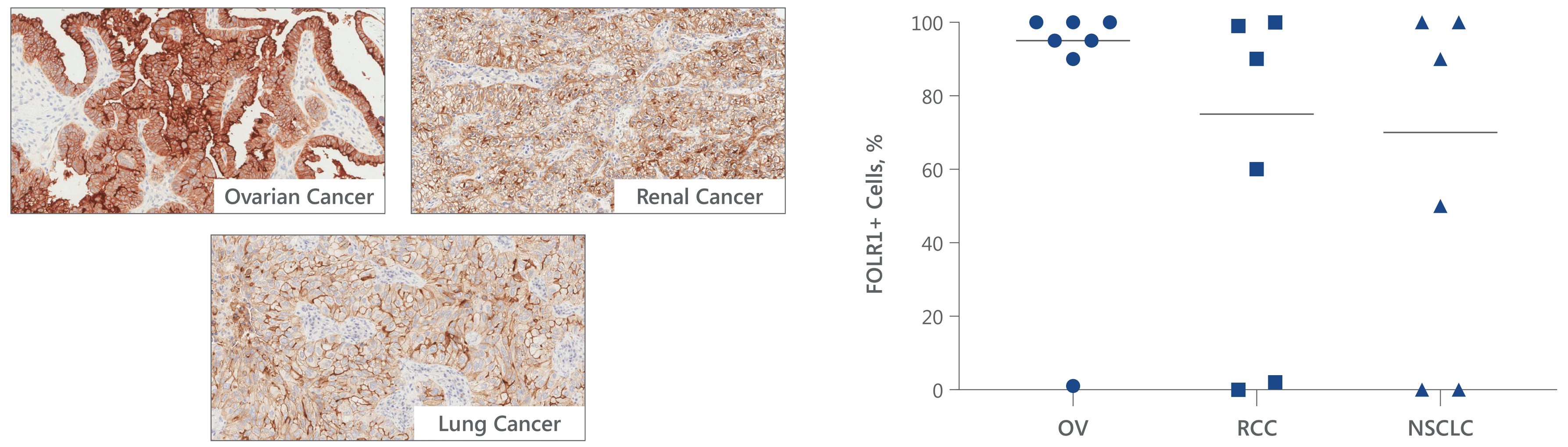
CoStAR, costimulatory antigen receptor; EC, extracellular; FOLR1, folate receptor alpha; IC, intracellular; MHC, major histocompatibility complex; scFv, single-chain variable fragment; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

METHODS

- A CoStAR molecule encoding an extracellular folate receptor alpha (FOLR1)-targeting single-chain fragment variable (scFv) and intracellular CD28 and CD40 signaling sequences was transduced with a lentiviral vector into peripherally harvested T cells from 3 healthy donors and TILs derived from 5 primary ovarian tumors
- Anti-FOLR1 CoStAR-expressing T cells were cocultured with target cells expressing:
 - Membrane-anchored anti-CD3 antibody (OKT3) to provide Signal 1 through TCR/CD3 complex crosslinking
 - FOLR1 to provide potent costimulatory Signal 2 through CoStAR
- T-cell activity was measured by quantifying expression of activation markers, cytokine secretion, T-cell proliferation, and cytolytic activity compared with a non-transduced control

RESULTS

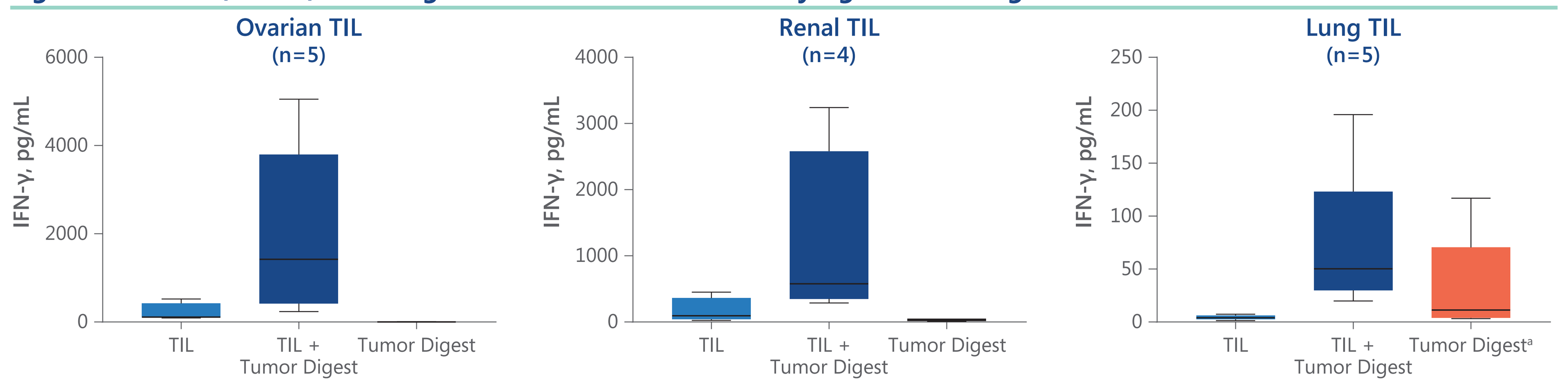
Figure 2. FOLR1 Protein Expression Profile in Selected Tumors


Ovarian Cancer
Renal Cancer
Lung Cancer
FOLR1+ Cells, %
OV
RCC
NSCLC

FOLR1, folate receptor alpha; NSCLC, non-small cell lung cancer (adenocarcinoma); OV, ovarian cancer (high-grade serous); RCC, renal cell carcinoma (clear cell).

- Robust protein expression of FOLR1 was observed in ovarian, renal, and lung cancer histologies

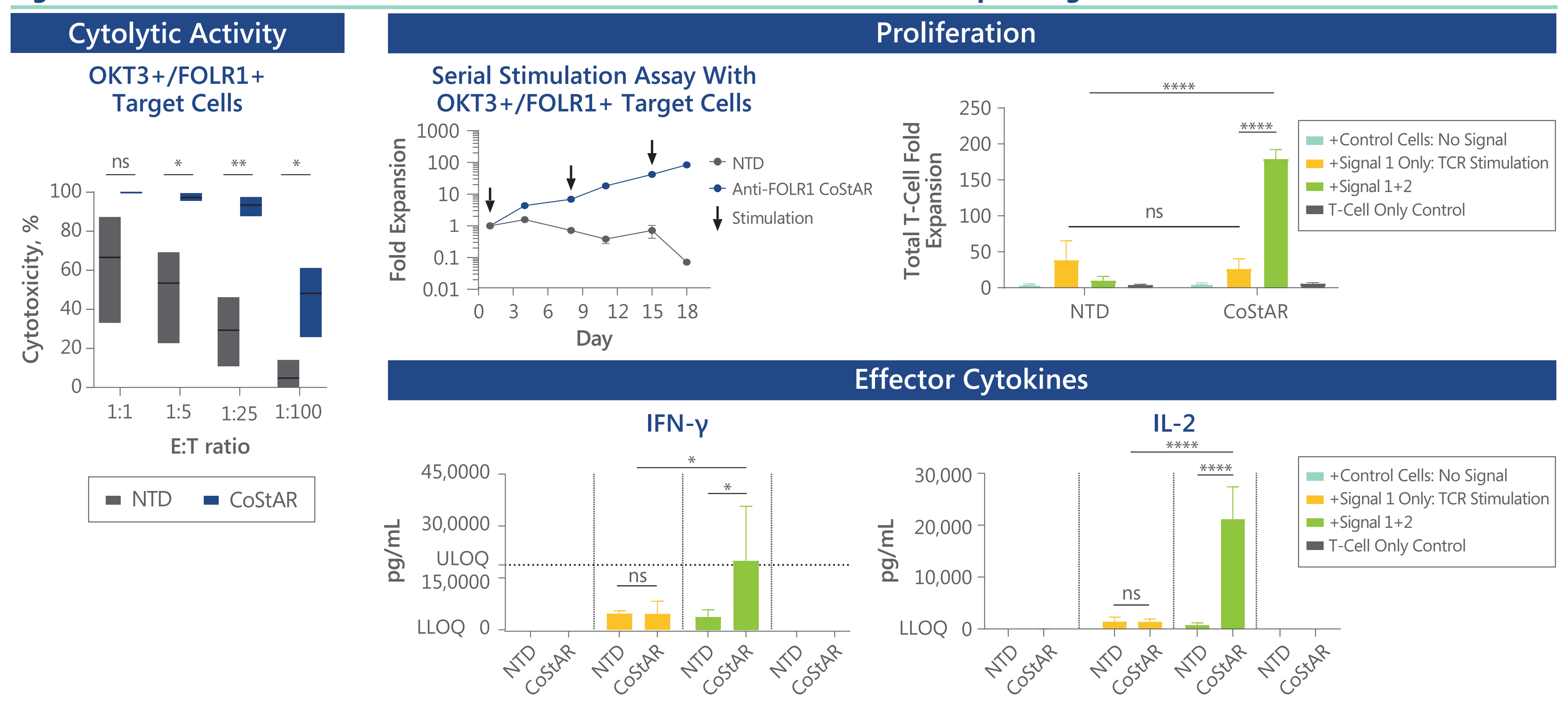
Figure 3. Ovarian, Renal, and Lung TILs Demonstrated Reactivity Against Autologous Tumor


Ovarian TIL (n=5)
Renal TIL (n=4)
Lung TIL (n=5)
IFN-γ, pg/mL
TIL
TIL + Tumor Digest
Tumor Digest
Tumor Digest[a]

[a] Lung tumor digests contain more T cells compared with other histologies, which results in background.
IFN, interferon; TIL, tumor-infiltrating lymphocyte.

- TILs isolated and manufactured from different tumor types showed interferon (IFN)-γ production when cocultured with ovarian, renal, and lung autologous tumor digests
- Comprehensive analysis in human T cells from 3 healthy donors and TILs isolated from 5 ovarian tumors are presented hereafter

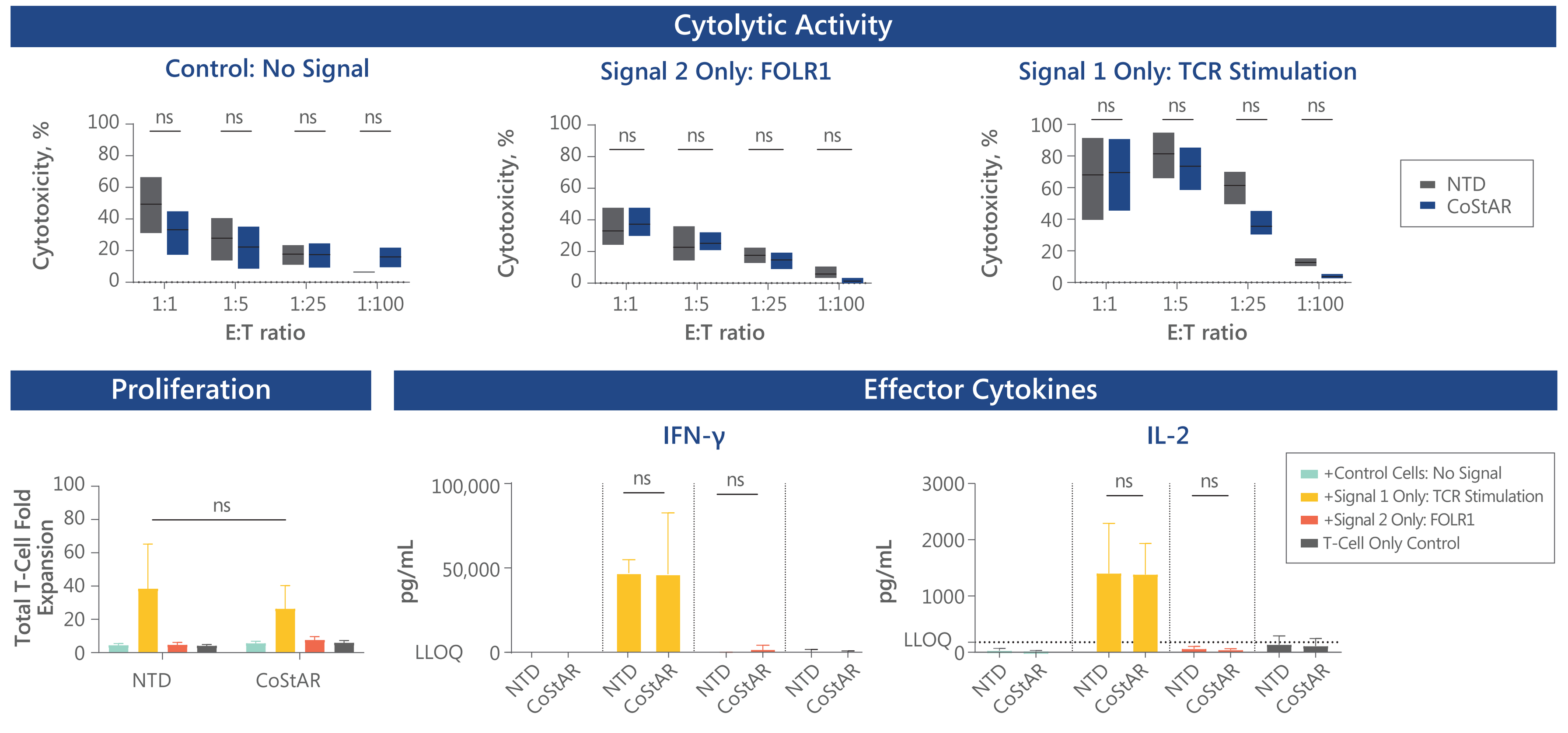
Figure 4. Marked Enhancement of Effector Function in Anti-FOLR1 CoStAR-Expressing T Cells


Cytolytic Activity
OKT3+/FOLR1+ Target Cells
Cytotoxicity, %
E:T ratio
NTD
CoStAR
Proliferation
Serial Stimulation Assay With OKT3+/FOLR1+ Target Cells
Fold Expansion
Day
NTD
Anti-FOLR1 CoStAR
Stimulation
Total T-Cell Fold Expansion
+Control Cells: No Signal
+Signal 1 Only: TCR Stimulation
+Signal 1+2
T-Cell Only Control
Effector Cytokines
IFN-γ
IL-2
pg/mL
ULOQ
LLOQ

Statistical significance was assessed by 2-way analysis of variance test; ns, $P>.1$, *$P<.0275$, **$P<.005$, and ****$P<.0001$.
CoStAR, costimulatory antigen receptor; E:T, effector:target; FOLR1, folate receptor alpha; IFN, interferon; IL, interleukin; LLOQ, lower limit of quantitation; ns, not significant; NTD, non-transduced; TCR, T-cell receptor; ULOQ, upper limit of quantitation.

- Healthy donor T cells were transduced with anti-FOLR1 CoStAR and cocultured with target cells expressing membrane-bound OKT3 with or without FOLR1
- CoStAR-expressing T cells performed equivalently to non-transduced T cells when cocultured with target cells that did not express FOLR1
- A dramatic improvement was seen in cytolytic activity, proliferation, and cytokine secretion by anti-FOLR1 CoStAR-expressing T cells compared with non-transduced T cells

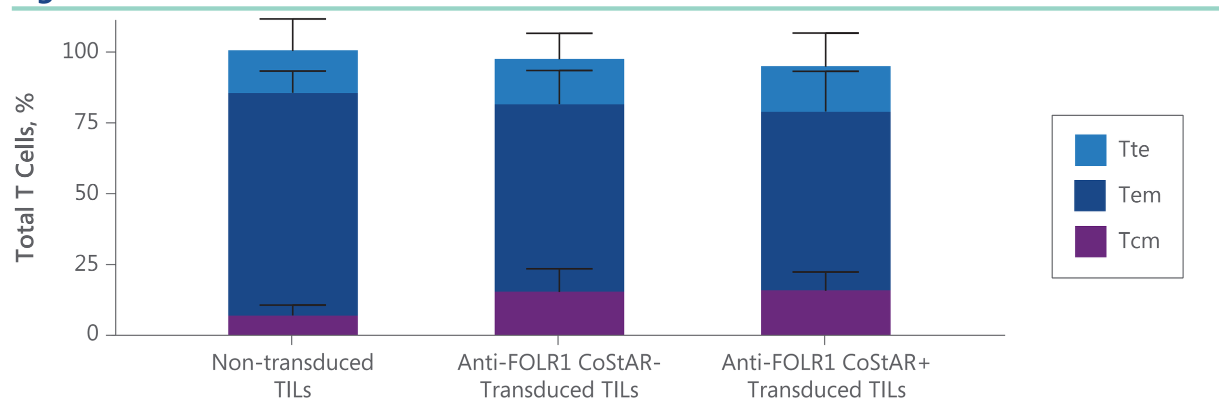
Figure 5. Binding of FOLR1 by CoStAR Alone Does Not Trigger Activation


Cytolytic Activity
Control: No Signal
Signal 2 Only: FOLR1
Signal 1 Only: TCR Stimulation
Cytotoxicity, %
E:T ratio
NTD
CoStAR
Proliferation
Total T-Cell Fold Expansion
Effector Cytokines
IFN-γ
IL-2
pg/mL
LLOQ
+Control Cells: No Signal
+Signal 1 Only: TCR Stimulation
+Signal 2 Only: FOLR1
T-Cell Only Control

Statistical significance was assessed by 2-way analysis of variance test; ns, P>.9.
CoStAR, costimulatory antigen receptor; E:T, effector:target; FOLR1, folate receptor alpha; IFN, interferon; IL, interleukin; LLOQ, lower limit of quantitation; ns, not significant; NTD, non-transduced; TCR, T-cell receptor.

- Healthy donor T cells were transduced with anti-FOLR1 CoStAR and cocultured with target cells expressing membrane bound FOLR1 or OKT3 alone
- Anti-FOLR1 CoStAR-expressing T cells demonstrated no increased effector activity over non-transduced T cells when exposed to FOLR1+/OKT3- target cells
- Anti-FOLR1 CoStAR-expressing T cells retained equivalent effector function compared with non-transduced T cells when exposed to FOLR1-/OKT3+ target cells

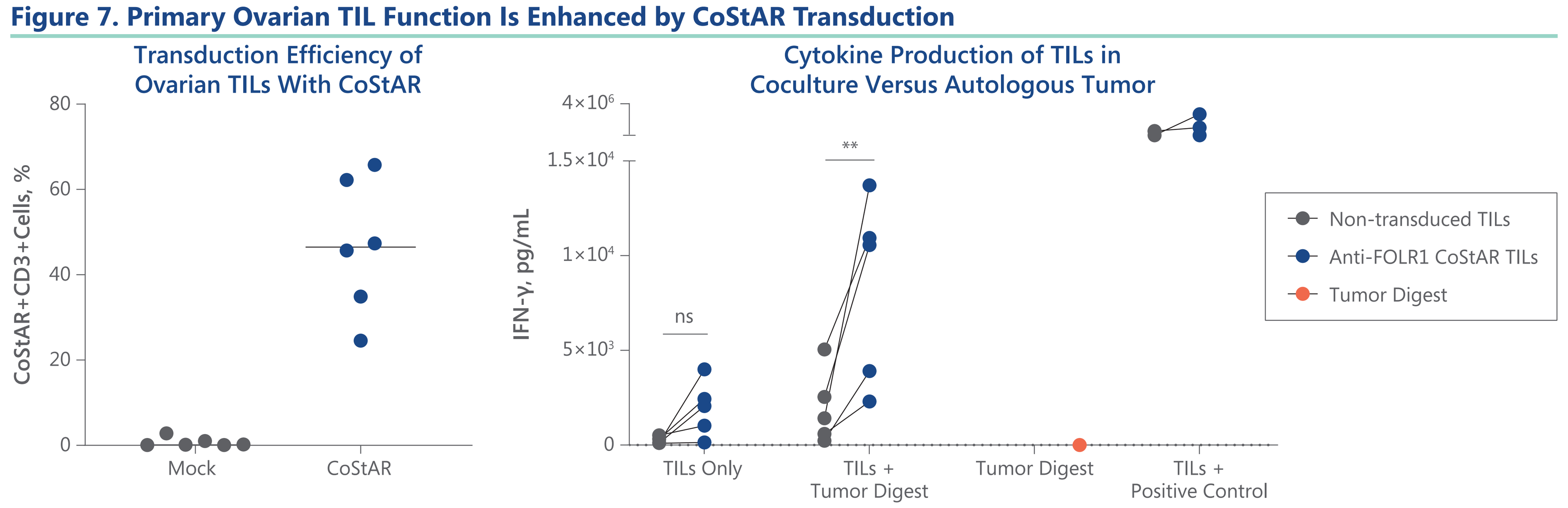
Figure 6. CoStAR Transduction Does Not Alter TIL Differentiation Status


Total T Cells, %
Non-transduced TILs
Anti-FOLR1 CoStAR- Transduced TILs
Anti-FOLR1 CoStAR+ Transduced TILs
Tte
Tem
Tcm

- The phenotype of ovarian TILs, as characterized by differentiation status, was not affected by anti-FOLR1 CoStAR modification

CCR, C-C chemokine receptor; CoStAR, costimulatory antigen receptor; FOLR1, folate receptor alpha; Tcm, central memory T cells (CD45RO+CCR7+CD95+CD27+); Tem, effector memory T cells (CD45RO+CCR7–CD95+CD27+/-); TIL, tumor-infiltrating lymphocyte; Tte, effector T cells (CD45RA+CCR7–CD27–CD95+).

Figure 7. Primary Ovarian TIL Function Is Enhanced by CoStAR Transduction


Transduction Efficiency of Ovarian TILs With CoStAR
CoStAR+CD3+Cells, %
Mock
CoStAR
Cytokine Production of TILs in Coculture Versus Autologous Tumor
IFN-γ, pg/mL
TILs Only
TILs + Tumor Digest
Tumor Digest
TILs + Positive Control
Non-transduced TILs
Anti-FOLR1 CoStAR TILs
Tumor Digest

Statistical significance was assessed by 2-way analysis of variance test; ns, $P>.2$ and **$P<.005$.
Phorbol 12-myristate 13-acetate (PMA) and ionomycin were used as a positive control.
CoStAR, costimulatory antigen receptor; FOLR1, folate receptor alpha; IFN, interferon; ns, not significant; TIL, tumor-infiltrating lymphocyte.

- Primary ovarian TILs were efficiently transduced with anti-FOLR1 CoStARs
- Improved cytokine secretion was observed when primary ovarian anti-FOLR1 CoStAR TILs were cocultured with autologous tumor digest

CONCLUSIONS

- The novel costimulatory antigen receptor anti-FOLR1 CoStAR molecule improves T-cell effector function through targeted costimulation upon engagement of tumor-expressed FOLR1
- When combined with concomitant TCR-specific binding, CoStAR's dual intracellular CD28 and CD40 signaling motifs significantly augment cytolytic activity, T-cell proliferation, and cytokine secretion
- Signaling through CoStAR alone does not trigger T-cell effector function
- Anti-FOLR1 CoStAR-expressing T cells retain normal function in the absence of FOLR1 expression on target cells
- Primary human ovarian TIL function is improved by CoStAR
 - For additional information on the CoStAR platform, please see poster 199 by Sykorova et al, titled "Potent T-Cell Costimulation Mediated by a Novel Costimulatory Antigen Receptor With Dual CD28/CD40 Signaling Domains to Improve Adoptive Cell Therapies"
- A first-in-human clinical study with ITIL-306, an investigational anti-FOLR1 CoStAR TIL product, is planned (sponsored by Instil Bio, Inc)
- Additional scFv targets are being evaluated for clinical application across a broad range of solid tumor histologies

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DISCLOSURES

SS, MK, MM, Y(S)O, GK: employment with and stock or other ownership in Instil Bio, Inc. **CY, EG, RA-R:** employment with and stock or other ownership in Instil Bio, Inc; and pending patent titled, "Methods of Isolating Tumor-Infiltrating Lymphocytes and Uses Thereof." **JSB:** employment with and stock or other ownership in Cellular Therapeutics, Immetacyte, Inc, and Instil Bio, Inc. **REH:** employment with and stock or other ownership in Instil Bio, Inc; and consultancy or advisory role for Anaveon AG, Novalgene, Ltd, Servier, Celyad, Celgene, Oxford Biomedica, GSK, Bristol Myers Squibb, Gilead, EUSA Pharma, Novartis, and Pfizer.

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