A 5T4 x CD3 Bispecific DART® Molecule with Extended Half-life for T-cell Immunotherapy of Cancers

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Abstract

Introduction: 5T4 (trophoblast glycoprotein), an oncofetal antigen involved in embryo development, is expressed on the cell surface of multiple cancers. Several 5T4-directed interventions have been reported, including a cancer vaccine and an antibody-drug conjugate. A superantigen-driven redirected cell killing modality was also developed, but obstacles, such as immunogenicity, remain to a successful 5T4 therapeutic molecule. Using the DART platform, we have developed a bispecific Fc-bearing 5T4 x CD3 protein designated “5T4 x CD3 DART”, designed to redirect T cells to target 5T4-expressing tumors.

Methods: 5T4 x CD3 DART, a humanized Fc-bearing DART molecule, was stably expressed in CHO cells and purified to homogeneity via a standard antibody-purification platform. In vitro characterization and functional studies were performed with 5T4-positive tumor cell lines and human T cells. In vivo studies were performed in immune-deficient tumor-bearing mice co-implanted with activated human T cells or reconstituted with human peripheral blood mononuclear cells (PBMCs). Pharmacokinetic studies were performed in both human FcRn transgenic mice and cynomolgus monkeys.

Results: 5T4 x CD3 DART demonstrated bispecific binding properties to both human and cynomolgus monkey antigens. In redirected cytolysis studies, 5T4 x CD3 DART-mediated lysis of 5T4-positive pancreatic, lung, renal, triple-negative breast, and ovarian cancer cell lines with EC_{50} values ranging between 0.03 and 0.08 ng/mL. 5T4 x CD3 DART displayed favorable pharmacokinetics with a prolonged circulating half-life in human FcRn transgenic mice and cynomolgus monkeys. Tumor clearance studies in NOD/SCID mice implanted subcutaneously with activated human T cells and tumor cells demonstrated robust inhibition of tumor growth upon intravenous (IV) administration of 5T4 x CD3 DART at doses as low as 0.8 µg/kg, but not with a CD3-binding control DART protein. In addition, human PBMC-reconstituted NOD/SCID/IL2rγ chain null mice were implanted intradermally with renal and pancreatic tumor cell lines or orthotopically with a triple negative breast cancer line and, after tumor establishment, were treated with 5T4 x CD3 DART. Antitumor activity was observed at doses as low as 4 µg/kg. Immunohistochemical analysis of tumor xenografts confirmed tumor clearance was associated with T-cell recruitment into the tumor mass.

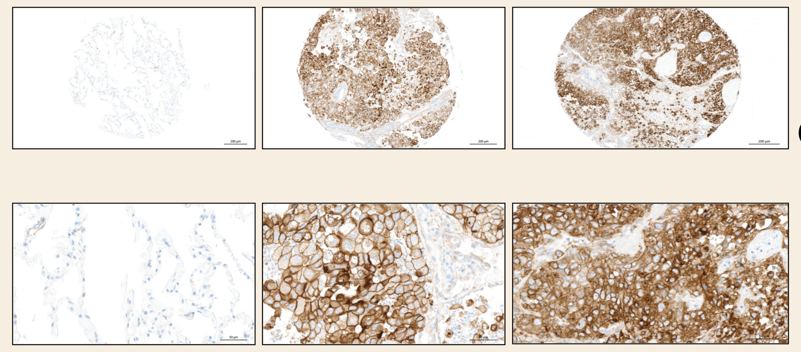
Conclusion: In summary, 5T4 x CD3 DART displays robust antitumor activity against several cancer cell lines in vitro and in vivo together with a favorable pharmacokinetic profile and merits further consideration as a potential treatment for 5T4-positive cancers.

Introduction

- 5T4 (trophoblast glycoprotein), an oncofetal antigen involved in embryo development[1], is expressed on the cell surface of multiple cancers with limited expression in normal adult tissues[2]
- Several 5T4-directed interventions have been evaluated clinically, including a cancer vaccine and an antibody-drug conjugate
- A superantigen-driven redirected cell killing modality was also developed[3], but was thwarted by immunogenicty
- Using the DART platform, we have developed a bispecific Fc-bearing 5T4 x CD3 protein designated “5T4 x CD3 DART”, designed to redirect T cells to target 5T4-expressing tumors

Expression of 5T4 in Cancer Tissues

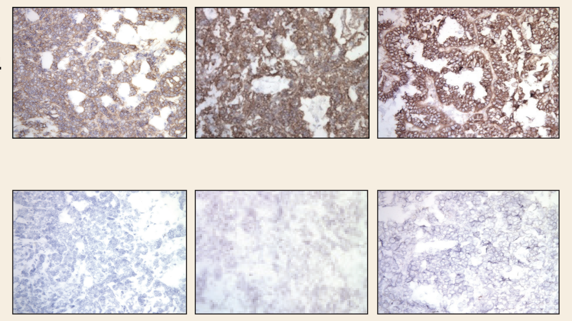
A.



Normal Lung
Lung Adenocarcinoma
Lung Squamous Cell Carcinoma

Positivity (membrane only)
Adenocarcinoma: 22/38 (58%)
Squamous: 31/37 (84%)

B.

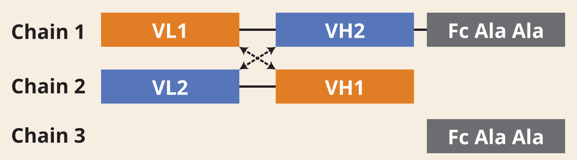


Breast Cancer
Renal Carcinoma
NSCLC Adenocarcinoma
Anti-5T4 (MGA5)
Isotype Control

A. IHC of FFPE tissues on independent TMAs with anti-5T4 Ab EPR5529 (abcam®) confirmed favorable normal tumor differential and high penetrance of expression in lung cancer.
B. Evaluation of a panel of anti-5T4 mAbs generated at MG demonstrating exhibited broad tumor reactivity identified MGA5, the parental mAb humanized and incorporated into the 5T4 x CD3 DART.

5T4 x CD3 DART

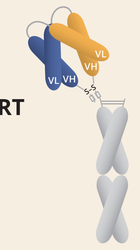
A.



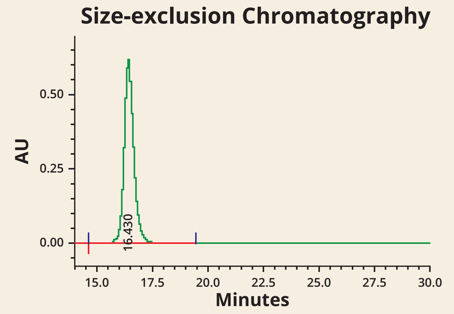
Chain 1
VL1
VH2
Fc Ala Ala
Chain 2
VL2
VH1
Chain 3
Fc Ala Ala

B.

5T4 x CD3 Fc-bearing DART
MW ~105 kDa

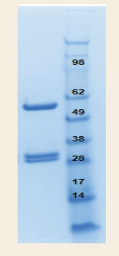


C.



Size-exclusion Chromatography
AU
16.430
Minutes

D.



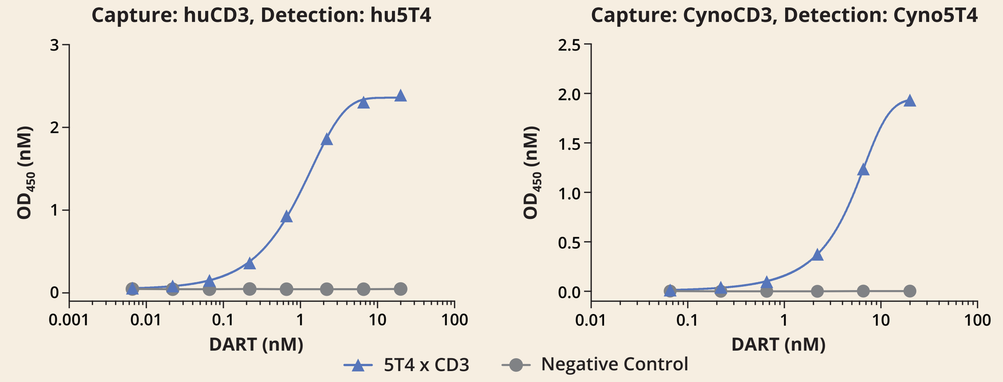
Reduced SDS-PAGE

- Humanized anti-5T4 and anti-CD3 variable domains were assembled through disulfide-linked heterodimer to form the DART protein
- Modified Fc, lacking effector function, was introduced for half-life improvement
- DART protein was generated in CHO cells
- Purified protein was subjected to SEC and reduced SDS-PAGE

Results

Binding Properties of 5T4 x CD3 DART

A.



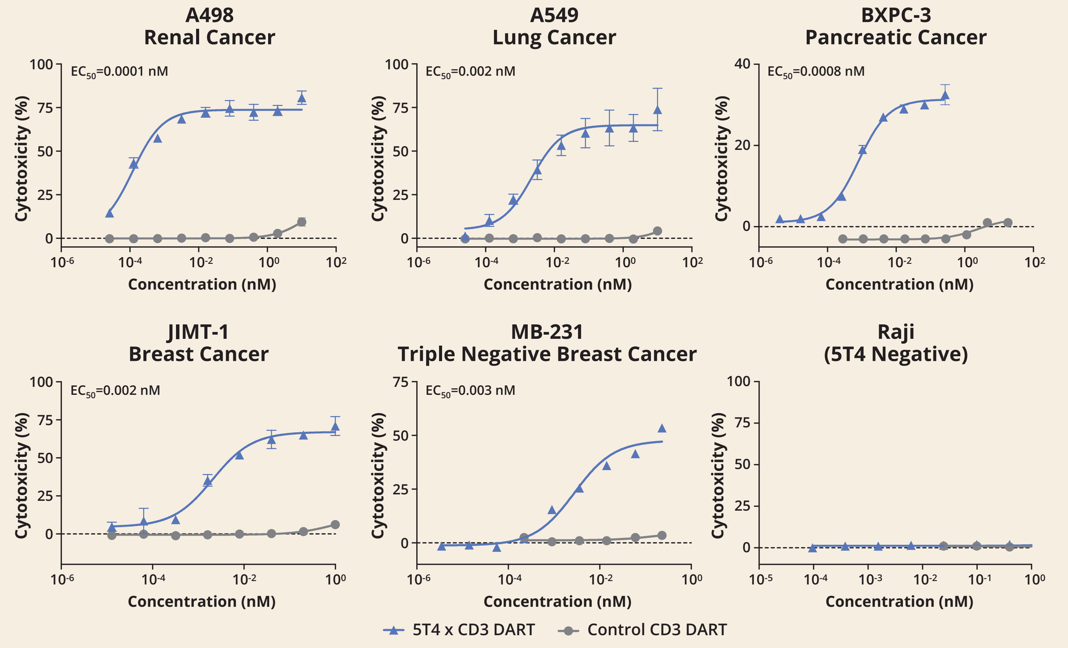
Capture: huCD3, Detection: hu5T4
Capture: CynoCD3, Detection: Cyno5T4
OD450 (nM)
DART (nM)
5T4 x CD3
Negative Control

B.

Binding of 5T4 x CD3 DART to Human and Cynomolgus Monkey CD3 and 5T4			
Antigen	Association Rate Constant (k_a) ($M^{-1}S^{-1}$)	Dissociation Rate Constant (k_d) (S^{-1})	Equilibrium Dissociation Constant (K_D) (nM)
Human CD3	2.1×10^5	2.6×10^{-3}	12.4
Cyno CD3	1.9×10^5	2.2×10^{-3}	11.6
Human 5T4	3.5×10^6	1.0×10^{-3}	0.29
Cyno 5T4	2.5×10^6	15×10^{-3}	6.0

A. Bispecific ELISA demonstrates simultaneous engagement of both target antigens by 5T4 x CD3 DART.
B. Equilibrium dissociation constants (K_D) for the binding of 5T4 x CD3 DART to human and cynomolgus (Cyno) monkey CD3 and 5T4 determined by surface plasmon resonance analysis.

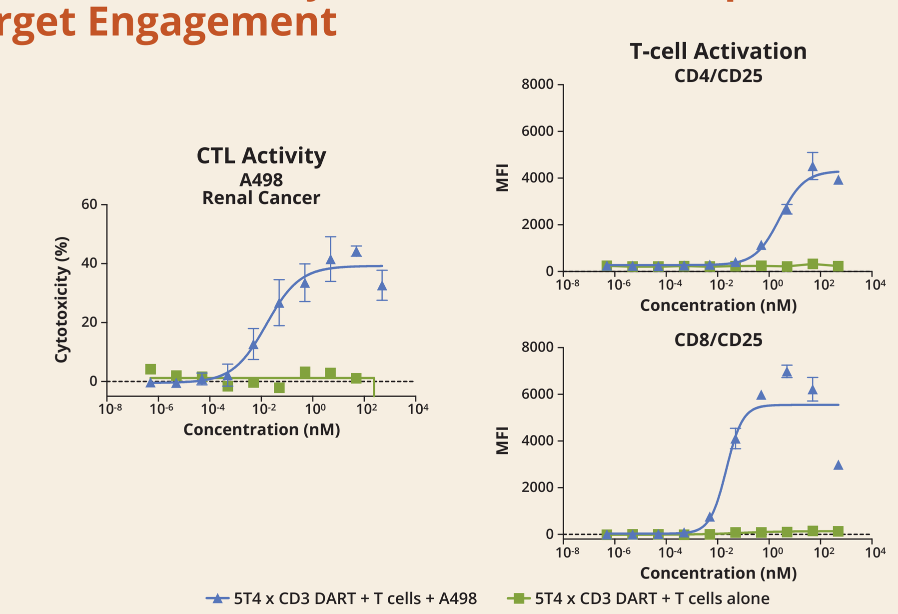
5T4 x CD3 DART-mediated CTL Activity Against Tumor Cell Lines



A498 Renal Cancer
EC_{50}=0.0001 nM
A549 Lung Cancer
EC_{50}=0.002 nM
BXPC-3 Pancreatic Cancer
EC_{50}=0.0008 nM
JIMT-1 Breast Cancer
EC_{50}=0.002 nM
MB-231 Triple Negative Breast Cancer
EC_{50}=0.003 nM
Raji (5T4 Negative)
Cytotoxicity (%)
Concentration (nM)
5T4 x CD3 DART
Control CD3 DART

Redirected cell killing (LDH method) by 5T4 x CD3 DART against 5T4-positive cancer cells. Experiments performed with human T cells (E:T=10:1) incubated for 24 hours.

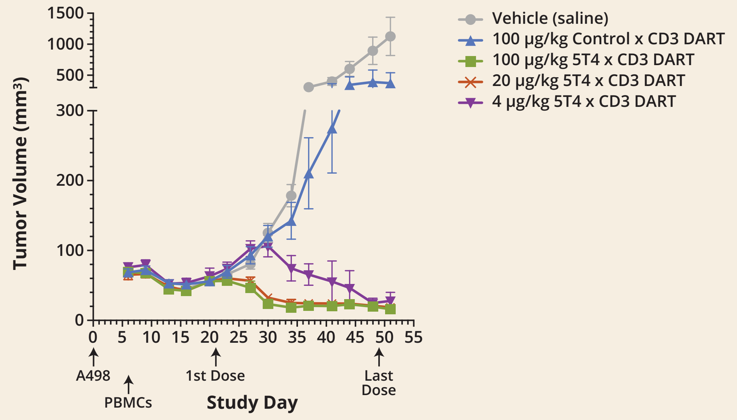
T-cell Activation by 5T4 x CD3 DART Requires Target Engagement



CTL Activity
A498
Renal Cancer
Cytotoxicity (%)
T-cell Activation
CD4/CD25
CD8/CD25
MFI
Concentration (nM)
5T4 x CD3 DART + T cells + A498
5T4 x CD3 DART + T cells alone

Redirected cytotoxicity (left panel) and T-cell activation (right panels) by 5T4 x CD3 DART in the absence or presence of target cells (Renal carcinoma cells A498). Human primary T cells were incubated with 5T4 x CD3 DART in the presence or absence of target cells (E:T=5:1) for 24 h. T-cell activation markers were measured by FACS analysis. Cytotoxicity was determined by LDH release assay.

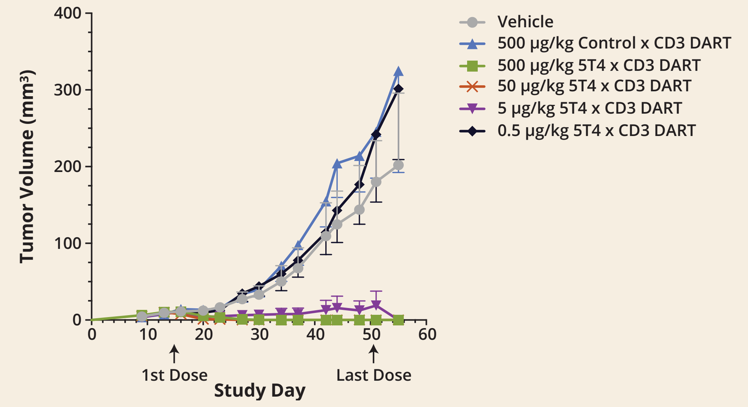
Regression of Established A498 RCC in Human PBMC-reconstituted Mice



Vehicle (saline)
100 µg/kg Control x CD3 DART
100 µg/kg 5T4 x CD3 DART
20 µg/kg 5T4 x CD3 DART
4 µg/kg 5T4 x CD3 DART
Tumor Volume (mm³)
A498
PBMCs
1st Dose
Last Dose
Study Day

NOD/SCID mice (n=8/group) were injected with tumor cells on Day 0 (intradermal, A498 cells 5 x 10[6]), reconstituted with PBMCs (10 x 10[6]) on Day 6 (IP), and treated as indicated starting on Day 21 (IV, Q4Dx9 schedule).

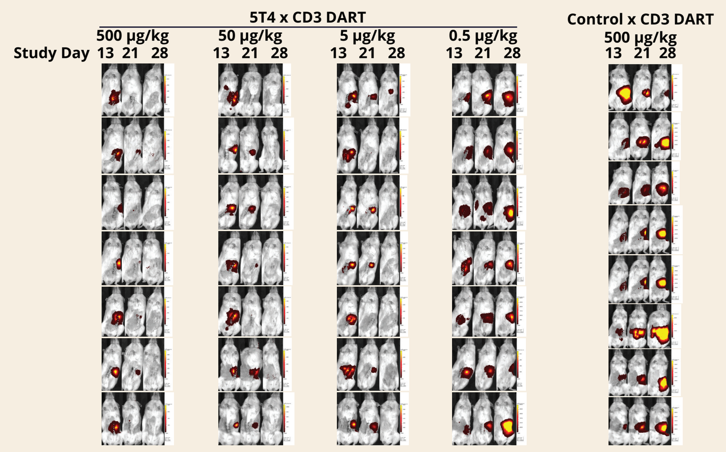
Antitumor Activity in an Orthotopic TNBC Model



Vehicle
500 µg/kg Control x CD3 DART
500 µg/kg 5T4 x CD3 DART
50 µg/kg 5T4 x CD3 DART
5 µg/kg 5T4 x CD3 DART
0.5 µg/kg 5T4 x CD3 DART
Tumor Volume (mm³)
1st Dose
Last Dose
Study Day

MHCI[-/-] mice were reconstituted with PBMCs (10 x 10[6]) (IP) and implanted orthotopically with TNBC MB-231 cells (1 x 10[6]) (OT) on Day 0. Following randomization, mice (n=7/group) were first treated (IV) on Day 15 and at weekly intervals thereafter for a total of 6 doses.

Antitumor Activity in an Orthotopic Pancreatic Cancer Model

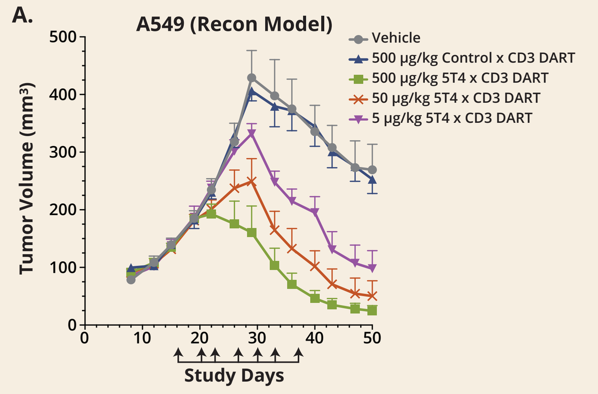


5T4 x CD3 DART
Control x CD3 DART
Study Day
500 µg/kg
50 µg/kg
5 µg/kg
0.5 µg/kg
13 21 28

MHCI[-/-] mice were reconstituted with PBMCs (10 x 10[6]) (IP) on Day 0 and implanted BXPC3 cells (1 x 10[6]) (OT) on Day 8. Following randomization, mice (n=7/group) were first treated (IV) on Day 15 and at weekly intervals thereafter for a total of 6 doses.

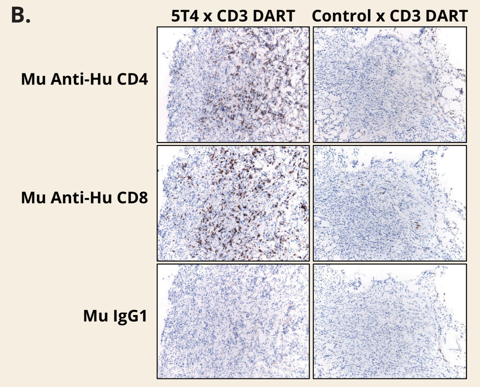
5T4 x CD3 DART Promotes T-cell Recruitment into Lung Cancer Mass

A.



A549 (Recon Model)
Vehicle
500 µg/kg Control x CD3 DART
500 µg/kg 5T4 x CD3 DART
50 µg/kg 5T4 x CD3 DART
5 µg/kg 5T4 x CD3 DART
Tumor Volume (mm³)
Study Days

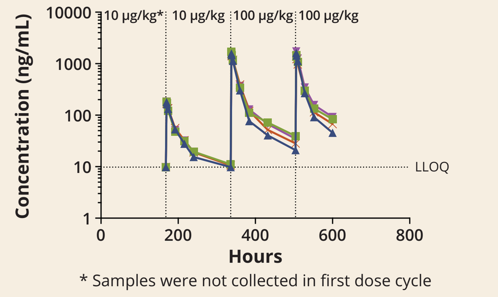
B.



5T4 x CD3 DART
Control x CD3 DART
Mu Anti-Hu CD4
Mu Anti-Hu CD8
Mu IgG1

A. B2M[-/-] mice (n = 7/group) were reconstituted with PBMCs (10 x 10[6]) (IP) on Day 0 and implanted A549 lung cancer cells (5 x 10[6]) (ID) on Day 1. 5T4 x CD3 or control DART was administered IV every 3 or 4 days for total 7 doses starting on Day 16 (arrows).
B. IHC using mouse anti-human CD4 or CD8 mAb in A549 lung cancer mass from mice treated with 5T4 x CD3 (50 µg/kg) or control (500 µg/kg) DART (**A**) after 72 hours.

5T4 x CD3 DART Displays Prolonged PK in Cynomolgus Monkeys



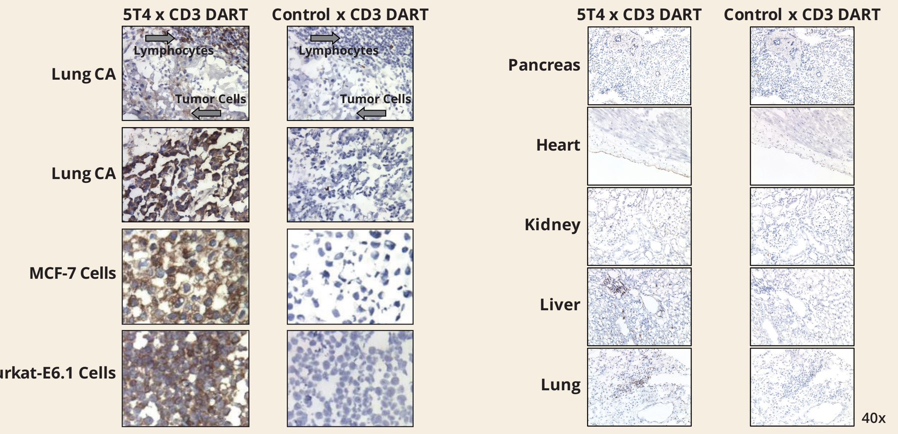
10 µg/kg*
10 µg/kg
100 µg/kg
100 µg/kg
Concentration (ng/mL)
LLOQ
Hours

* Samples were not collected in first dose cycle

PK Parameters (Mean ± SEM) (10 µg/kg)	
Clearance	1.7 ± 0.52 mL/h/Kg
Terminal $t_{1/2}$	4.3 ± 0.04 days
MRT	4.0 ± 0.58 days

- 4 weekly doses with dose escalation at 3rd and 4th week of 5T4 x CD3 DART via 2-hour IV infusion were well tolerated
- Drug exposure increased proportionally with dose escalation

Tissue Cross-reactivity of 5T4 x CD3 DART



5T4 x CD3 DART
Control x CD3 DART
Lung CA
Lymphocytes
Tumor Cells
MCF-7 Cells
Jurkat-E6.1 Cells
Pancreas
Heart
Kidney
Liver
Lung
40x

- 5T4 x CD3 DART exhibits strong tumor vs. normal tissue reactivity

Conclusions

- The oncofetal trophoblast glycoprotein 5T4 is overexpressed in a range of tumors
 - 5T4 shows limited adult tissue expression with strong tumor/normal differential
- A bispecific Fc-bearing 5T4 x CD3 DART was generated to specifically target T cells to 5T4-expressing tumors
- 5T4 x CD3 DART exhibited:
 - Dose-dependent redirected cell killing against a wide range of solid tumor cell lines in vitro
 - T–cell activation strictly dependent on co-engagement of 5T4 x CD3 DART with 5T4-expressing target cells
 - Efficacy in multiple established tumor models (renal, pancreatic, and triple negative breast cancer) in human PBMC-reconstituted mice
 - 5T4 x CD3 DART promoted T-cell recruitment to tumor xenograft in mouse model
 - 4 weekly doses with dose escalation up to 100 µg/kg were well tolerated in cynomolgus monkeys
 - Extended circulating half-life in nonhuman primates (~4.3 days) supports feasibility of convenient clinical dosing

These data support further investigation of 5T4 x CD3 DART as a potential candidate for the treatment of 5T4-expressing cancers

References

1. Ward, C. M. J. Cell Sci. (2003), 116:4533-4542. **2.** Southall, P.J. et. al. Br. J. Cancer (1990), 61: 89-95.
3. Eisen, T. et. al. Curr Oncol Rep (2014) 16:370.

Acknowledgment

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