In Vivo Efficacy and Mechanism of Action of anti-TIGIT Monoclonal Antibody CASC-674

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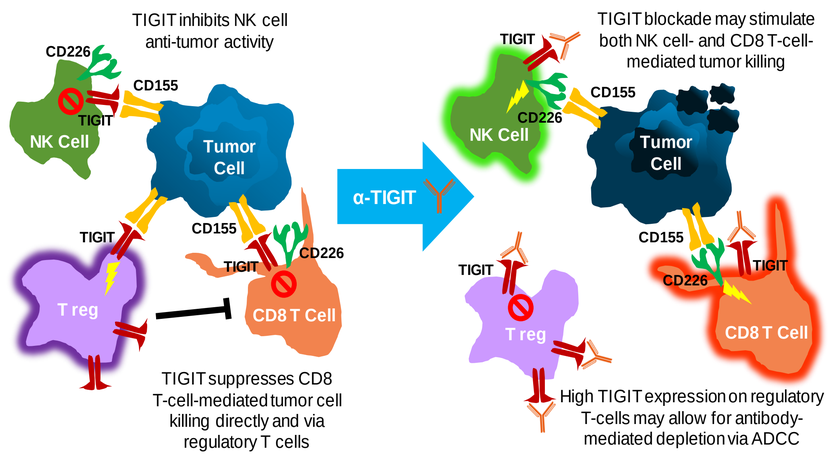
Background

- TIGIT (T cell immunoreceptor with Ig and ITIM domains) is a co-inhibitory immune receptor expressed on subsets of activated, memory and regulatory T cells and NK cells
  - Upregulated on tumor infiltrating immune cells and associated with dysfunctional $CD8^+$ tumor infiltrating lymphocytes
  - Co-expressed with exhaustion markers such as PD-1, TIM-3 and LAG-3
  - TIGIT expressing regulatory T cells (Tregs) display a highly suppressive phenotype
- TIGIT ligands include CD155 (poliovirus receptor [PVR]- high affinity) and CD112 (PVRL2 - lower affinity)
  - Expressed on dendritic cells, macrophages and a variety of tumor tissues e.g. stomach, skin, lung
  - Also bind to often co-expressed costimulatory receptor CD226 with lower affinity than to TIGIT
- The TIGIT-CD226-CD155-CD112 network modulates adaptive and innate immune responses
- In collaboration with Adimab Inc. a panel of fully human antagonistic monoclonal antibodies that bind with sub-nanomolar affinity to human, NHP and mouse TIGIT was identified that block receptor-ligand interactions and signaling in T cells *(Piasecki et al., AACR, 2017)*
- From this panel, a lead candidate was selected, CASC-674, with good developability based on biophysical and biochemical properties
- Here we report on the *in vivo* mechanism of action and efficacy of CASC-674 in a number of syngeneic mouse tumor models

Therapeutic Rationale for Targeting TIGIT in Cancer



CASC-674 Shows Good Developability Characteristics

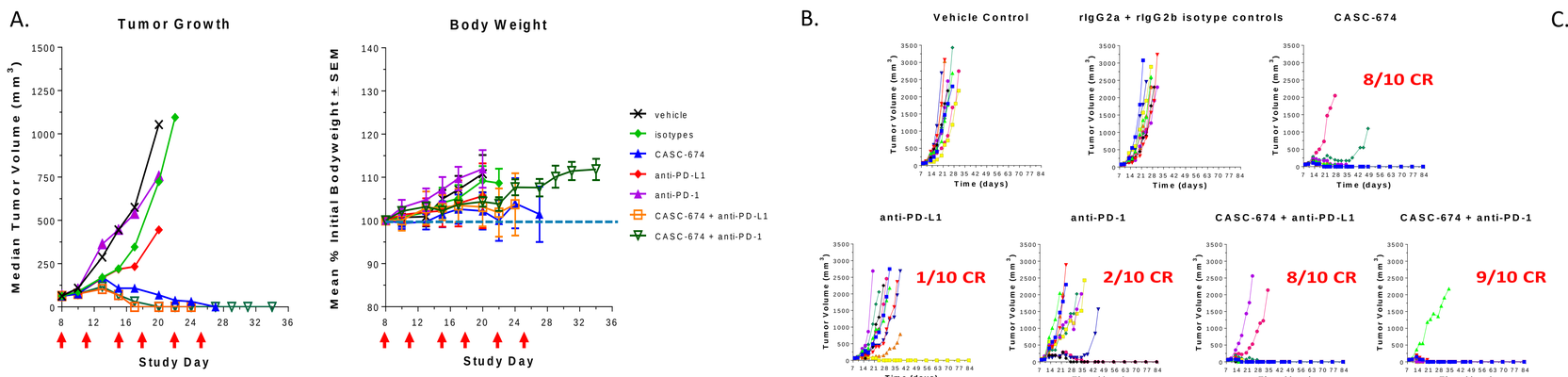
- Biophysical Characterization (HEK293 produced human IgG1)

PSR Score	HIC retention time (min)	AC-SINS	Tm[1] (°C)	Monomeric[2] (%)	HEK293 Titer[3] (mg/L)
0.00	10.7	2.2	78.0	97.3	162

[1]Tm: Fab Tm determined from IgG sample. Fab Tm = 1st unfolding event after the CH2 peak at approx. 58°C
[2]Determined by size exclusion chromatography after protein A purification
[3]Titer from transient expression in HEK293 cells

- Biochemical Characterization after stress conditions
  - Oxidation: 0.1% H_2O_2, 24h. Fd, Fc and light chain detection of oxidation status. Tryptic peptide mapping to confirm site(s) and correlate with predicted surface exposed methionine
  - Isomerization: pH 5.5, 40°C, 2 weeks. Fd, Fc and light chain detection of Asu (-18Da) species. Tryptic peptide mapping to confirm site(s)
  - Deamidation: pH 8.5, 40°C, 1 week. Ides digestion and Fc removal, followed by CEX to detect acidic shift species. Tryptic peptide mapping to confirm site(s)
- CASC-674 and its affinity matured progeny do not undergo chemical modification under stress and have favorable biophysical characteristics

CASC-674 Shows Potent Single Agent Activity in a CT26 Tumor Model and Leads to Long-term Tumor Free Survivors

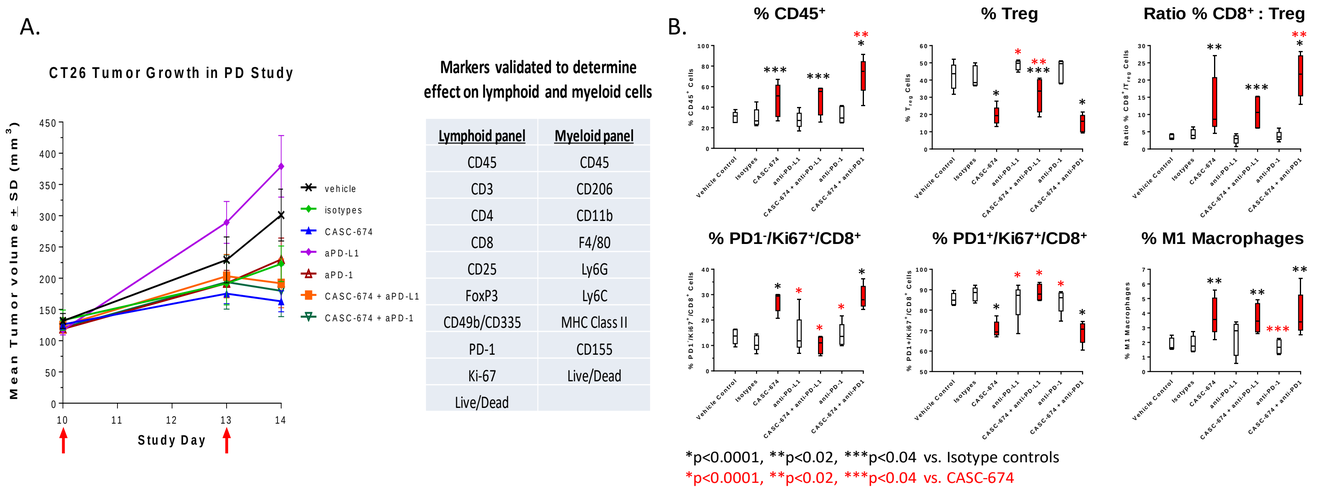


TGD (days)	% TFS (day 83)	% TFS CT26 (day 113)	% TFS 4T1 (day 113)
0	0	NA	
2.1	0	NA	
>59.2	80	100	100
5.4	10	100	100
7.1	20	100	0
>59.2	80	100	62.5
>59.2	90	100	100

→ **Treatment of original CT26 tumors resulted in CT26 specific T cell memory in all groups**

Figure 1. A. Anti-tumor efficacy and effect on body weight in a CT26 syngeneic colon carcinoma tumor model. Groups of 10 female BALB/c mice were inoculated with CT26 cells in the right flank. BIW, IP treatment (arrows) began when the mean tumor volume was 67 ± 9 mm^3. Mice were treated with CASC-674 (mIgG2a version) at 5 mg/kg/dose, anti-PD-L1 (10F.9F2, rIgG2b) or anti-PD-1 (RMP1-14, rIgG2a) at 10 mg/kg/dose either alone or in combination. B. Individual tumor volumes in each treatment group until the end of the experiment (day 83). CR: complete responders. C. Percentage of mice in each treatment group with tumors less than the upper limit of 2000 mm^3. Tumor free survivors (TFS, CRs in B.) were re-challenged on day 83 with twice the amount of CT26 cells as the original inoculum on the opposite site as the original inoculum and with 4T1 breast cancer cells on the other flank. Development of tumors was followed for 30 days. TGD = median time (days) in treatment group – median time (days) in control group to reach a tumor volume of 2000 mm^3.

CASC-674 Changes the CT26 Tumor Microenvironment from an Immunosuppressive to an Inflammatory Phenotype

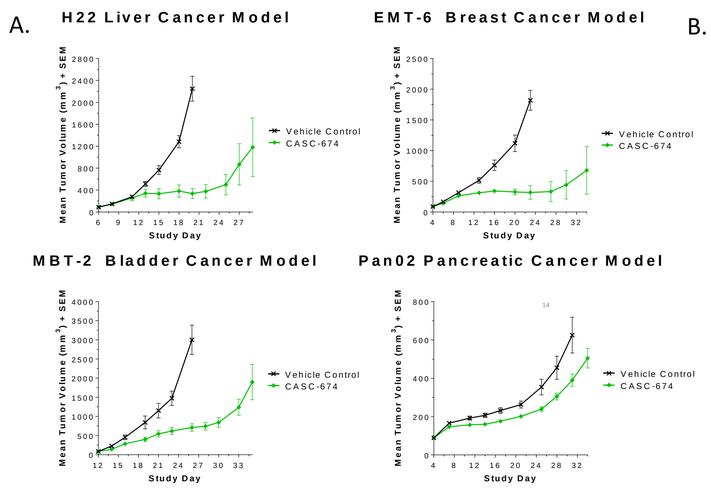


Markers validated to determine effect on lymphoid and myeloid cells

Lymphoid panel	Myeloid panel
CD45	CD45
CD3	CD206
CD4	CD11b
CD8	F4/80
CD25	Ly6G
FoxP3	Ly6C
CD49b/CD335	MHC Class II
PD-1	CD155
Ki-67	Live/Dead
Live/Dead	

Figure 2. A. Groups of 5 BALB/c mice were inoculated with CT26 cells in the right flank. Treatment commenced when the mean tumor volume was 125 ± 22 mm^3 and was administered IP on days 10 and 13. Mice were treated with the same agents at the same concentrations as in Figure 1. Tumors and spleens were collected on day 15 and processed for flow cytometric analysis of various lymphocyte and myeloid cell populations. Cells were stained with antibodies against the markers listed. The T/NK panel allows for the enumeration of the % leukocytes, pan T cells, helper T cells, cytotoxic T cells, regulatory T cells (Tregs), exhausted and non-exhausted cytotoxic T cells, NK and NK-T cells. The myeloid panel allows for the enumeration of the % leukocytes, M1 and M2 macrophages and Granulocytic and Monocytic-Myeloid Derived Suppressor Cells. B. Whisker plots showing the percentage positive cells in each treatment group and p values (one-way ANOVA SAS v9.4) relative to isotype controls and CASC-674 by itself. No statistically significant (P<0.05) changes in these same cell populations were observed in the spleen.

CASC-674 Shows Robust Single Agent Activity in a Variety of Tumor Models



B.

Tumor Model	Number of doses	% TGI
MC38	6	28 %
EMT-6	5	87 %
Pan02	6	43 %
CT-26	6	97 %
A20	4 (vehicle) 6 (CASC-674)	27 %
LL/2	6	23 %
RM-1	4 (vehicle) 5 (CASC-674)	14 %
Renca	4 (vehicle) 5 (CASC-674)	11 %
MBT-2	4 (vehicle) 6 (CASC-674)	79 %
B16-F10	3 (vehicle) 4 (CASC-674)	-9 %
H22	5 (vehicle) 6 (CASC-674)	89 %
B16-BL6	4 (vehicle) 5 (CASC-674)	3 %

Figure 3. CASC-674 was tested as a single agent in a MuScreen (CrownBio) in 12 different syngeneic tumor models of 9 different cancer types and the effect compared to Vehicle (PBS) control. Mice were administered PBS or CASC-674 at 5 mg/kg/dose, IP, BIW x 3 for a total of 6 doses unless the tumors reached the upper size limit before that. Treatment started when the mean tumor volume was approximately 90 ± 10 mm^3. A. Mean tumor volumes for complete groups (n=8 mice/group). B. Tumor Growth Inhibition (TGI) obtained with CASC-674 for all 12 models tested. % TGI= 1-[mean tumor volume in CASC-674 group on last day – mean tumor volume in CASC-674 group on first day / mean tumor volume in vehicle group on last day – mean tumor volume in vehicle group on first day] x 100.

Summary

- Cascadian Therapeutics has identified novel, high-affinity (K_D <10 - <100 pM), cross reactive, fully human TIGIT antibodies with good developability properties that block TIGIT binding and function
- Lead development candidate: CASC-674
  - Shows potent single agent activity in a CT26 CRC model and in other syngeneic tumor models such as: breast (EMT-6), bladder (MBT-2), liver (H22) and pancreatic cancer (Pan02)
  - Changes the CT26 tumor but not the spleen microenvironment from an immunosuppressive state to an inflammatory anti-tumor phenotype, while anti-PD-1 or anti-PD-L1 treatment alone has a marginal effect on the tumor immune-phenotype
  - The effect of CASC-674 on the tumor microenvironment was enhanced in combination with anti-PD1 treatment and to a lesser extent with anti-PD-L1 treatment
- The observed (single agent) activity; unique human, NHP and murine cross reactivity; high affinity and good developability characteristics support the development of CASC-674 as a therapeutic candidate

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