Evaluate pharmacodynamic and patient enrichment biomarkers for SAR444881, a first-in-class anti-ILT2 monoclonal antibody for cancer immunotherapy

Ilana Mandel[*1], Rui Wang[*2], Anne Caron[3], Eladio Marquez[2], Colette Dib[3], Donald Jackson[2], Cecilia Deantonio[4], Dana Haves Ziv[1], Ilana Goldshtein[1], Tsuri Peretz[1], Dror Alishekevitz[1], Sharon Hashmueli[1], Yair Sapir[1], Tehila Ben Moshe[1], Giovanni Abbadessa[2], Itay Friedman[#1], Nigel Crawford[#2]

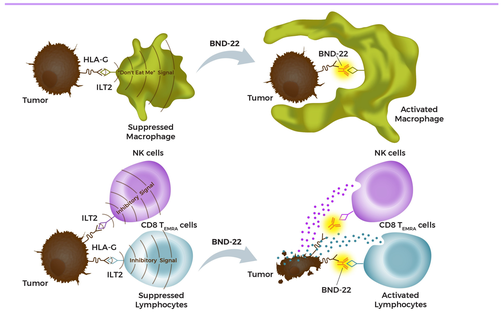
[1]Biond Biologics, Misgav, Israel; [2]Sanofi, Cambridge, MA, USA; [3]Sanofi, Paris, France; [4]Sanofi, Cambridge, UK

*Co-first Authors; #Co-correspondence

INTRODUCTION

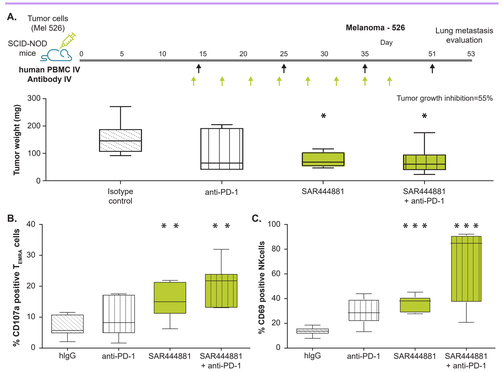
- Leukocyte Ig-like receptor B1 [LILRB1; immunoglobulin-like transcript 2 (ILT2)] is an inhibitory receptor expressed on various immune cells, shown to bind to classical and nonclassical MHC class I molecules, with the highest affinity to HLA-G.
- ILT2-mediated inhibition leads to impairment of immune cell proliferation, differentiation, phagocytosis, cytotoxicity and cytokine secretion, and thus antagonism of the ILT2 signalling axis may serve as a potential novel target for anti-cancer immunotherapy.
- SAR444881 (BND-22) is a novel humanized IgG4 monoclonal antagonist antibody that selectively binds to ILT2 and blocks its interaction with MHC I molecules.
- Studies showed that SAR444881 demonstrates robust macrophage and lymphocyte-driven anti-tumor activity in in vitro and in vivo models.[1]
- To overcome the limitation that LILRB proteins are not expressed in rodents, we conducted a series of in vivo studies using humanized mouse models, cancer patient biopsies and ex vivo co-culture systems to interrogate the pharmacodynamic (PD) response of ILT2 antagonism as well as inform the combinatorial and patient enrichment strategies for SAR444881.

Figure 1. Mechanism of action of SAR444881 (BND-22)



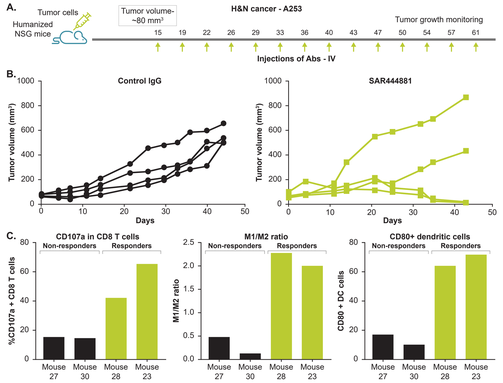
RESULTS

Figure 2. In vivo anti-tumor activity of SAR444881 is associated with increase in activation markers in lymphocytes



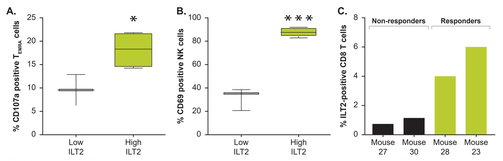
SCID-NOD mice were engrafted intravenously (IV) with MEL526 cells. Human PBMCs isolated from healthy donors were administered to the mice together with the relevant treatments starting from day 14 according to the treatment schedule indicated in the illustration (**A**). **B.** The levels of CD107a in T$_{EMRA}$ cells and **C.** CD69 in NK cells in the lungs of the treated mice. Un-paired T-test was used to compare the groups, * P<0.05 ** P<0.005 *** P<0.0005.

Figure 3. In vivo anti-tumor activity of SAR444881 is associated with changes in the TME



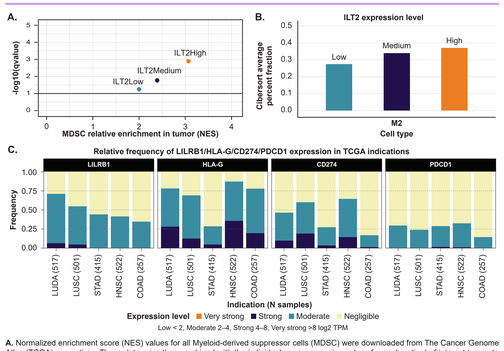
A. Human CD34+cells-engrafted mice were inoculated with A253 cells. Mice were treated at indicated treatment schedule with SAR444881 or a control IgG when tumors reached 80mm³. **B.** Tumor growth in mice inoculated with CD34+ cells from one donors treated with either SAR444881 or control IgG4. **C.** On day 46 mice were sacrificed, and levels of different markers were evaluated by FACS analysis. CD107a in CD8 T cells, M1/M2 ratios, and levels of CD80+ dendritic cells in TME (tumor microenvironment) of the indicated mice are presented.

Figure 4. ILT2 levels correlate with response to SAR444881 in vivo models



CD107a (**A**) and CD69 (**B**) expression in SAR444881 treated T$_{EMRA}$ or NK cells in mice from **Fig.2** that received PBMC from donors with low or high levels of ILT2 in their T$_{EMRA}$ cells or NK cells, respectively. **C.** Baseline ILT2 levels in peripheral CD8 T cells of indicated mice from **Fig.3**.

Figure 5. ILT2 gene expression in the TME is correlated with enrichment of suppressive myeloid populations in different cancer indications



A. Normalized enrichment score (NES) values for all Myeloid-derived suppressor cells (MDSC) were downloaded from The Cancer Genome Atlas (TCGA) consortium. These data were then combined with the individual gene expression values for each patient of interest to create a data set to analyze individual cell type enrichment stratified by gene-level expression. A plot of MDSC enrichment in patients with low, medium, and high ILT2 levels is presented. **B.** Cibersort was used to analyze cell fractionation for M2 cell types analyzed across patients expressing low, medium, and high ILT2 levels. An analysis of patients that contained above 25% quartile levels of CD8 was performed for each indication present in the TCGA. **C.** Profiling gene expression distribution of LILRB1 and related targets across various tumors from TCGA.

Figure 6A. ILT2 is predominantly expressed on immune cells in the TME of multiple tumor types

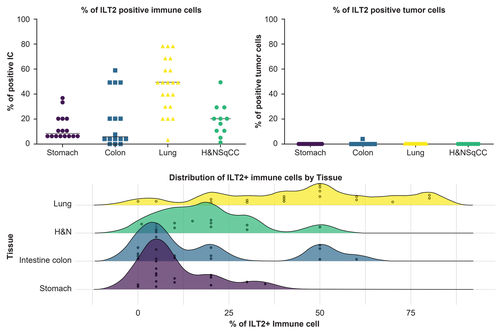
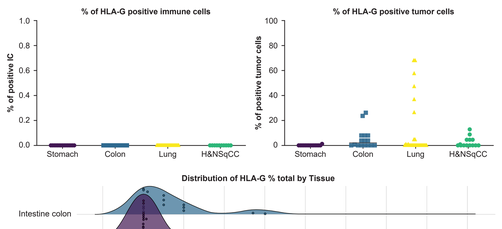
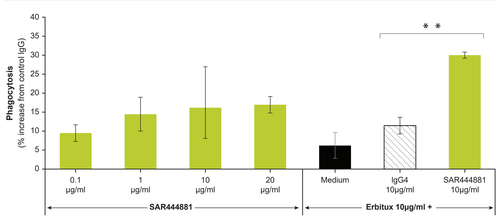


Figure 6B. HLA-G is predominantly expressed on tumor cells in multiple tumor types



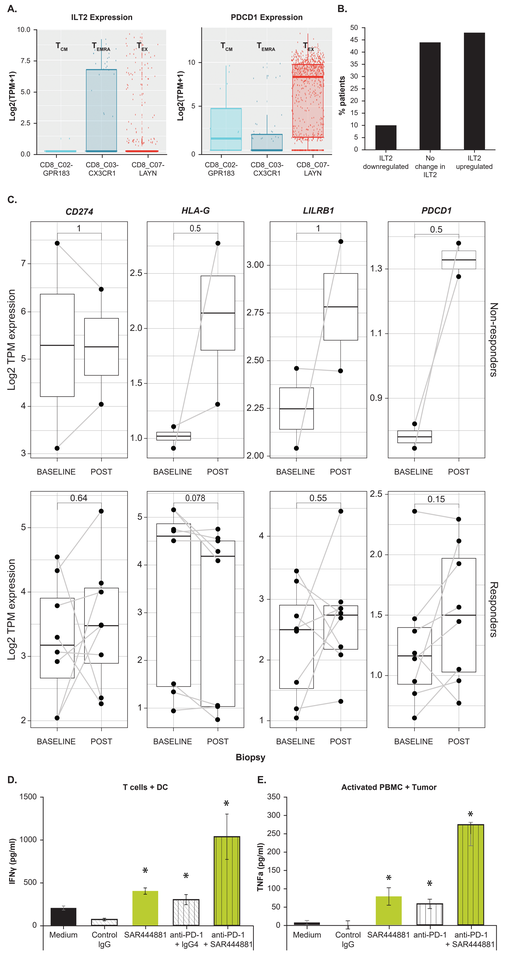
Metastatic tumor tissue collected from participants in MOSCATO 01 study has been profiled for (**A**) ILT2 (clone EPR21007) and (**B**) HLA-G (clone 1E5A10) expression using chromogenic single plex IHC. ILT2 positive immune cells were identified in all the tumor types profiled with ~10% of samples having ILT2+ immune cells ≥ 1% and with the highest detection in lung > HNSCC > Colon~Stomach. While ILT2 is not expressed on tumor cells, HLA-G is detected only on tumor cells at various levels with a rare detection in gastric cancers.

Figure 7. SAR444881 can be combined with tumor-targeting antibodies to increase anti-tumor activity



A253-HLA-G cells were stained with pHrodo Red Cell Labeling Dye, washed and added to macrophages generated from monocytes isolated from healthy donors, along with the various indicated treatments in triplicates. A single representative time point of the experiment at 9.5 hours is presented. The percent of phagocytosis increase compared to macrophages and target cells with control IgG is presented. ******P<0.01; un-paired Student's T-test compared to Erbitux + medium.

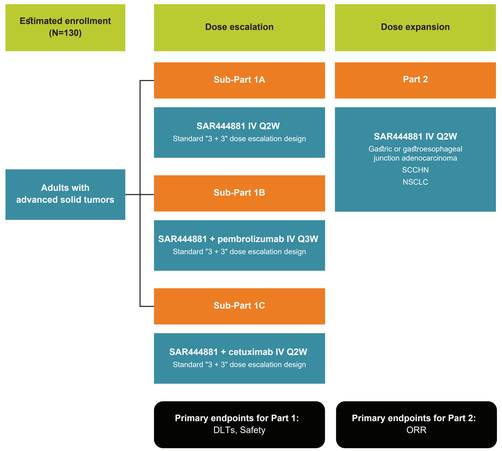
Figure 8. SAR444881 can be used in anti-PD-1 experienced patients or in combination with anti-PD-1



A. ILT2 and PD-1 are expressed by different CD8 T cell sub-populations. An analysis of ILT2 and PD-1 expression in intra-tumoral CD8 T cells from CRC patients documented in http://crotcell.cancer-pku.cn/. T$_{CM}$ – central memory T cells. T$_{EMRA}$ – T cells re-expressing CD45RA. T$_{EX}$ – exhausted T cells. **B.** ILT2 gene expression is increased in 48% melanoma patients after treatment with anti-PD-1. Bioinformatic analysis of ILT2 expression in melanoma patients treated with Nivolumab.[2] **C.** The RNAseq data were used to calculate gene expression changes of ILT2 in Non-small cell lung cancer patients treated with anti-PD-1 in responders and non-responders. log2 ILT2 fold-change differences above 0.5 or below 0.5 were regarded as increase and decrease in expression, respectively. **D.** Combining SAR444881 with anti-PD-1 has a synergistic effect on T cell anti-tumor activity. Dendritic cells (DC) were incubated with autologous CD8 T cells together with the indicated treatments. IFNg secretion was quantified by ELISA. **E.** Single-cell suspensions of a fresh tumor isolated from a colon cancer patient were incubated with PBMC generated from the same patient in the presence of the indicated treatments. *P<0.05; un-paired Student's T-test compared to control IgG.

Figure 9. First-in-human trial of SAR444881 (BND-22) is currently enrolling patients with advanced solid tumors

- A Phase 1/2, dose escalation and expansion study of the safety, tolerability, and anti-tumor activity of SAR444881 (BND-22) administered alone and in combination with other therapeutic agents (NCT04717375).
- SAR444881 is currently under investigation and its efficacy and safety have not been established.



DLTs, dose-limiting toxicities; IV, intravenous; NSCLC, non-small cell lung cancer; ORR, overall response rate; Q2W, every 2 weeks; Q3W, every 3 weeks; SCCHN, squamous cell carcinoma of the head and neck

CONCLUSION

- The present translational research data from murine models demonstrated pharmacodynamic modulation of activated CD8, NK cell and myeloid populations mediated by SAR444881.
- ILT2 level expression has been found to be associated with antitumor activities in various murine models. Further analysis revealed its gene expression is correlated with enrichment of suppressive myeloid populations in TME across a variety of cancer indications.
- The profiling results of ILT2 and HLA-G in several metastatic tumor types suggest their potential utility as a patient enrichment biomarker.
- Functional assessment suggests SAR444881 can be combined with cetuximab to improve the anti-tumor activity.
- ILT2 and PD-1 are expressed by different T cell populations. SAR444881 synergizes with anti-PD-1 in various preclinical systems. Expression of ILT2 and HLA-G is induced by treatment with anti-PD(L)-1 in patients with advanced malignancies. All this suggests ILT2 blockade may benefit anti-PD(L)1-experienced patients.
- An ongoing phase 1/2 trial of SAR444881 mono- and combination therapy in patients with advanced solid tumors is testing these concepts in the clinic (NCT04717375).

Acknowledgments

We want to thank Wenting Wang and Yingchun Liu of Sanofi for the contribution to figure output generation and Mukul Rastogi, PhD of Sanofi for the editorial support.

Disclosures

RW, AC, EM, CD, DJ, CD, GA, NC: Employed by Sanofi and may hold shares and/or stock options in the company.
IM, DH, IG, TP, DA, SH, YS, TBM, IF: Employed by Biond Biologics and may hold shares and/or stock options in the company.

Correspondence

Itay Friedman (Itay@biondbio.com) or Nigel Crawford (Nigel.Crawford@sanofi.com)

References

1. Mandel I, et al. *Cancer Res.* 2020; 80 (16_Supplement): 3266.
2. Riaz N, et al. *Cell.* 2017;171(4):934-949.e16.

ASCO JUNE 3-7 2022