OncoTAM, a comprehensive preclinical platform to explore Macrophages as key drivers of cancer progression and develop new therapies against Tumor-Associated-Macrophages

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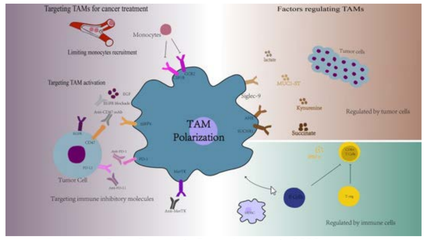
Introduction

Tumor-Associated Macrophages (TAMs) play an important role in tumor development, modulation of neoangiogenesis, immune suppression, and metastasis. A high infiltration of macrophages in the tumor is also often correlated with a poor prognosis in several types of cancer. Therefore, they became an attractive target for cancer immunotherapies. Several macrophage-targeting approaches in anticancer therapy are under development, including TAM depletion, inhibition of new TAM differentiation, or re-education of TAM activation for cancer cell phagocytosis.



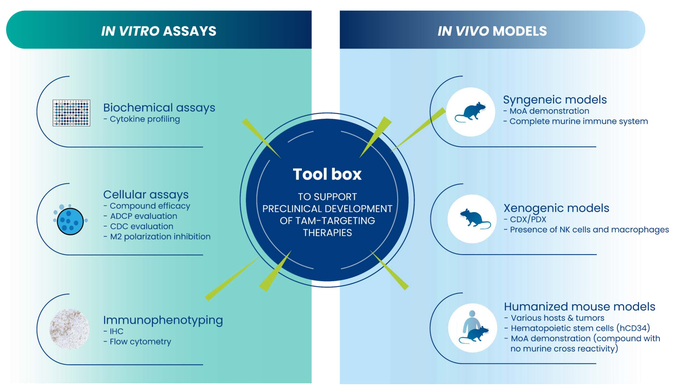
From Pan et al., Front. Immunol., 2020

In order to better evaluate and support the pre-clinical development of novel TAM-targeting strategies, we implemented a large panel of *in vitro* assays and *in vivo* models:

- Antibody-dependent cellular phagocytosis (ADCP) has been used to demonstrate a crucial mechanism of action of several antibody (Ab)-based therapies targeting macrophages.
- Some of these Ab (including anti-CSF1R) have been tested in syngeneic in vivo tumor models
- We demonstrated that the site of tumor implantation in mice (PAN-02, subcutaneous vs orthotopic) could impact the macrophage polarization (M1 vs M2). Differences in the ratio of M1 and M2 subtypes infiltrating the PAN-02 murine pancreatic tumors were observed
- Anti-CSF1R antibodies induced a slight reduction in the tumor mass of Renca murine kidney tumor by eliminating TAMs, but not in other syngeneic tumor models like MC38
- In an orthotopic Hepa1-6 murine liver cancer model, we showed high antitumor efficacy of compounds targeting the STAT6 pathway by reprogramming immunosuppressive TAMs into an M1 phenotype that promotes the induction of a cytotoxic immune response.
- For compounds displaying no cross-reactivity with murine target, we developed models and characterized the TAMs in breast, colon, melanoma and head & neck PDX and CDX tumors in different huCD34-engrafted mouse models.

Conclusion

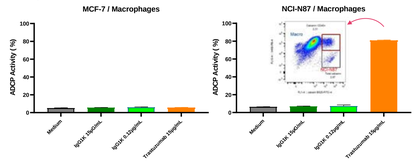
We designed a comprehensive preclinical platform to support the development of TAM-targeting therapies.



Experiments

In vitro assay for evaluating ADCP of antibodies

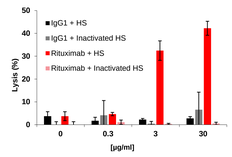
Trastuzumab induced antibody-dependent cell phagocytosis against Her2 positive NCI-N87 cells but not against Her2 negative MCF-7 cells (flow cytometry analysis).
This ADCP assay is available with human and murine macrophages or neutrophils



Monocytes isolation from human buffy coat and 7-day differentiation in hGM-CSF supplemented RPMI medium
Staining of target tumor cells with Calcein-AM, treatment with negative IgG1k or Trastuzumab (15 µg/ml) and co-culture with human macrophages (E/T ratio 25:1, 3h)

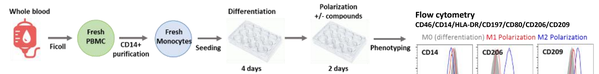
In vitro assay for evaluating CDC of antibodies

Rituximab induced complement dependent cytotoxicity (Raji cell line, ^{51}Cr release assay)



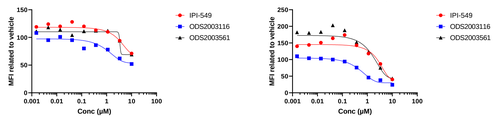
Loading of target Raji cells with 45 µg Na_2 $^{51}CrO_4$ solution Treatment with negative IgG1 or Rituximab (3 doses) and incubation with human serum, inactivated or not for 3h (spontaneous ^{51}Cr release evaluated by incubation with cell culture medium and maximum release by incubation with 4% Triton, X-100) .

In vitro M2 polarization inhibition assay

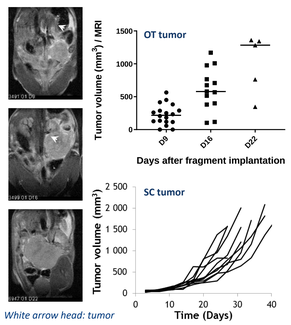


Phenotypic assay with M2 macrophage inhibitor

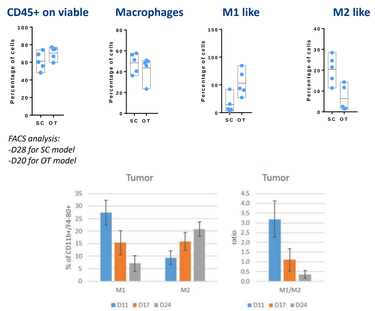
✓ **Impact of IPI-549 and 2 ODS compounds (PI3K inhibitors) on M2 polarization**



In vivo Subcutaneous *vs* orthotopic implantation of PAN02 pancreatic murine tumor impacts the polarization of tumor macrophages (M1 vs M2)

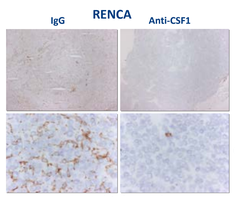


White arrow head: tumor



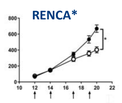
FACS analysis:
-D28 for SC model
-D20 for OT model

TAMs elimination by *in vivo* anti-CSF1R treatment in Renca murine kidney tumors or MC38 colon murine tumors

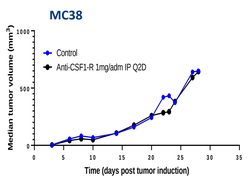


Tumor associated macrophages
F4/80 IHC in OT Renca tumor samples

Anti-CSF1R treatment show a slight anti-tumor efficacy in Renca tumors but did not show any efficacy in MC38 tumor model despite significant TAM depletion

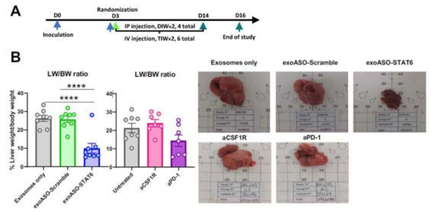


From O'Brien S. et al, Cancer Immunol, 2021
Only RENCA αCSF1R-treated tumors had a significant increase in neutrophil cell density, promoting CAF to secrete chemokines involved in neutrophil recruitment.
Not observed across all tumor models, potentially due to differences in fibroblast content



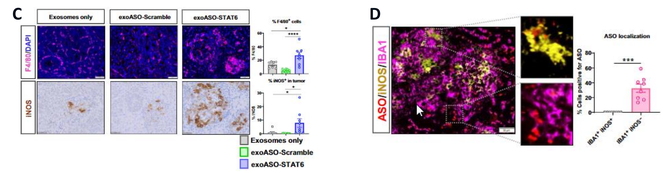
C57BL/6 mice bearing orthotopic Hepa1-6 tumors in the liver, treated with systemic administration of exoASO-STAT6 result in a potent monotherapy anti-tumor response

From Kamerkar S. et al., Sci Adv, 2022, in vivo work done at Oncodesign Services



exoASOSTAT6 results in effective re-programming to an M1 phenotype that promotes the induction of a cytotoxic immune response and an antitumoral TME

A. Experimental study design
B. Antitumoral efficacy as represented by liver weight (LW) vs body weight (BW) ratio of mice bearing Hepa1-6 tumors in the liver, IV treated with **exosomes** only, **exoASO-Scramble** (12mg)(TIW, 2weeks), or **exoASOSTAT6-2039** (PTGFRN++) (12mg)(TIW, 2 weeks) or IP with **anti-CSF1R** (10mg/kg)(DIW) or **anti–PD-1** (10mg/kg)(BIW).
C. Quantification of F4/80 (macrophage) and iNOS expression, performed by immunofluorescence and IHC (immunohistochemistry) respectively, in Hepa1-6 tumor sections
D. Representative images and quantification of ASO localization in iNOS-positive, IBA1+ (M1), and iNOS-negative, IBA1+ (M2), macrophages.



Immune-humanized mice to monitor the MOA of compounds on human macrophages

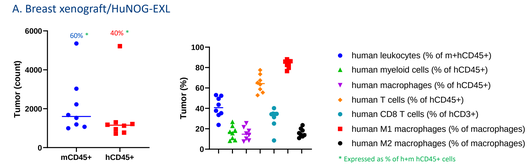
Myeloid lineage engraftment is enhanced by the use of immunodeficient mice expressing specific human cytokines such as NOG-EXL and BRGSF mice

Mouse host	Hu. Method	Premium features	Human Immune cells reconstitution T cell	B cell	Myeloid	NK cell
NOG-EXL	hHSC	• Express human GM-CSF and human IL-3 • Increased myeloid populations • Long term stable engraftment, no GvHD	●	●	●	○
BRGSF	hHSC	• hDC boost on demand by hFLT3L injection • Activity of complement (C5) • Suitable for infection & vaccination studies	●	●	●	○
NOG-FcγR$^{-/-}$	hHSC	• Lack expression of functional mouse FcγRs • Lack activity of endogenous ADCC • Suitable for assessment of antibody-based therapies	●	●	●	○

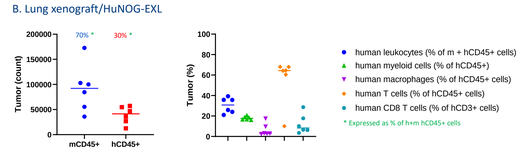
● *Optimal level of presence*
○ *Potential level of presence*

Immunophenotypical characterization of 2 xenograft models in hCD34-engrafted NOG-EXL or BRGSF mice

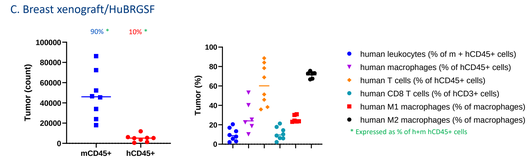
A. Breast xenograft/HuNOG-EXL



B. Lung xenograft/HuNOG-EXL



C. Breast xenograft/HuBRGSF



→ **Data demonstrated a moderately high frequency of macrophages within the tumor.**

→ **Immunophenotypical profile is tumor and host- dependent**

→ **Mouse immune cells, which are strickly from the myeloid lineage, represent the dominant myeloid population.**

→ **Others internal data indicate that immune cell repertoire is driven by the tumor**

All animal procedures were approved by the Animal Care and Use Committee of Oncodesign Services (Oncomet - CNREEA agreement N° 91)

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