Gastrointestinal/liver-targeted TLR7 agonist for treatment of colorectal and liver cancers

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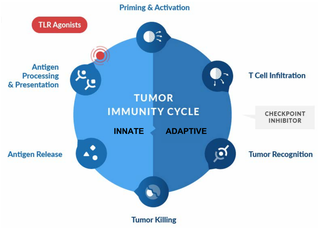
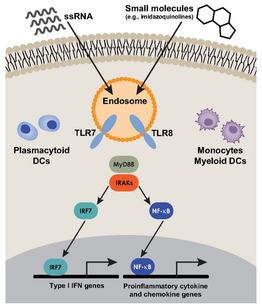
1. Introduction

- Checkpoint inhibitors (PD-1/PD-L1 antibodies) have become the backbone of IO therapies, however, only a fraction of patients respond
- Current data suggest response rates are influenced by a myriad of mechanisms affecting the tumor microenvironment, including mutational burden and level of immune infiltrate (ref 1)
- Innate immune agonists have the capacity to convert “cold” immune quiescent tumors into “hot” infiltrated tumors, thus providing a complementary mechanism to checkpoint blockade
- As clinical proof-of-concept, intratumoral TLR9 agonists have shown promising anti-tumor responses in combination with checkpoint inhibitors; however, successful clinical applications have been limited to cutaneous-accessible tumor types (ref 2)



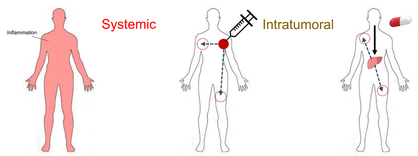
2. Toll-Like Receptor 7

- **Expression**: Human TLR7 expressed mainly in plasmacytoid dendritic cells (professional APC)
- **Function**: TLR7 activation elicits Type I interferon, upregulates costimulatory molecule (CD86), increases antigen processing/presentation (MHC), and drives greater T cell stimulation
- **Druggability**: One of the few innate immune receptors that can be activated by small molecule, which allows for fine tuning of ADME/PK properties using proven medchem design principles
- **Examples**: Intratumoral TLR7 agonists have demonstrated pre-clinical and early clinical efficacy in solid tumors (NKTR-262, MEDI-9197, LHC165)



Dowling, *ImmunoHorizons* **2018**, *2*, 185-197

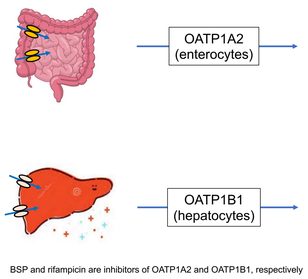
3. APR003 is a GI/Liver-Targeted Oral TLR7 agonist



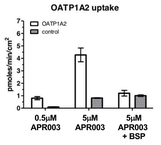
LOCAL IMMUNE PRIMING LEADS TO SYSTEMIC ANTI-TUMOR IMMUNITY

- **Systemic** administration leads to poor tolerability and potential immune tolerance induction
- **Intratumoral** administration affords local immune priming of tumor antigens leading to systemic (abscopal) immunity, but application limited to cutaneous accessible tumors
- **Oral GI/liver targeted** TLR7 agonist can circumvent the limitations of both systemic and intratumoral approaches to treat colorectal and liver cancers, and potentially other cancers with liver metastases

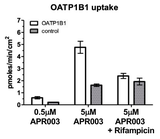
4. GI/Liver Targeting Through OATP



OATP1A2 (enterocytes)



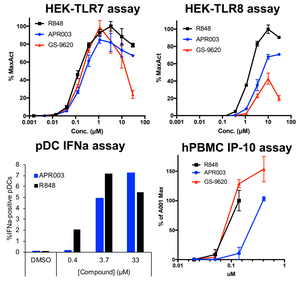
OATP1B1 (hepatocytes)



BSP and rifampicin are inhibitors of OATP1A2 and OATP1B1, respectively

- APR003 was designed using medicinal chemistry principles of liver-targeting drugs, including transporter uptake properties (ref 3)

5. In vitro Activity



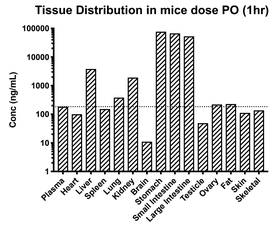
Cellular Assay	R848	GS9620	APR003
TLR7 EC_{50} (μM)	0.14	0.21	0.23
TLR8 EC_{50} (μM)	2	>30	4.4
THP1 (TLR2/4/8) EC_{50} (μM)	9.7	>30	>30
hu PBMC IP-10 LEC (μM)	0.05	0.05	0.14
mk PBMC IP-10 LEC (μM)	0.14	0.14	0.41
ms Spleno IP-10 LEC (μM)	<0.02	0.14	<0.02

- Effective concentration at 50% of maximal response (EC_{50}) are shown for sigmoidal dose response curves.
- Lowest effective concentration (LEC), defined as the lowest concentration of test compound that elicited at least 3-fold signal over vehicle control, are shown for bell-shaped dose response curves.

- **Target selectivity**: APR003 is >10-fold selective for TLR7 over TLR8
- **Cellular activity**: Active in plasmacytoid dendritic cells, but not in THP-1 monocytes
- **Species cross reactivity**: Active across mouse, monkey, and human cells

6. Pharmacokinetics

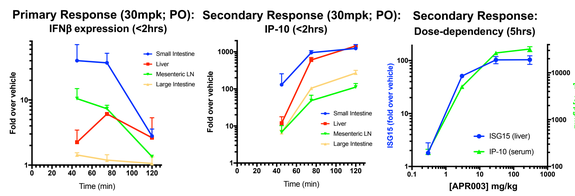
Route	PK Parameters	Mouse	Monkey
IV	Dose (mg/kg)	3	1
	$t_{1/2}$ (h)	2.92	1.38
	CL (mL/min/kg)	99.6	21.6
	V_{dss} (L/kg)	1.88	0.688
Oral	Dose (mg/kg)	10	5
	T_{max} (h)	0.25	0.58
	C_{max} (ng/mL)	94.2	104
	AUC_{0-t} (ng·h/mL)	76.5	178
	F (%)	4.6	4.2



- **Pulsatile kinetics (ideal for *in situ* vaccination)**: High clearance, short elimination half-life, and low volume of distribution
- **Tissue-restricted distribution**: concentrates in the GI, liver, and kidney (consistent with transporter profile)

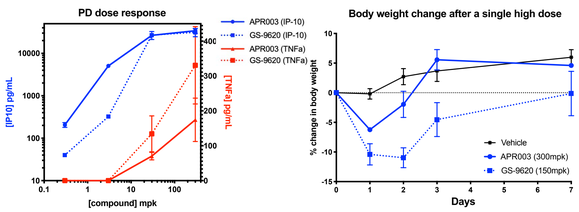
7. Pharmacodynamics

TLR7 activation → Type I IFN (transient) → IP-10, ISG (amplified)



- **Primary response (transient)**: IFNb expression elicited in GI, liver, mesenteric lymph node
- **Secondary response (amplified)**: interferon stimulated genes (ISG) in the target tissues and interferon-inducible protein 10 (IP-10) in serum
- **Dose dependency**: no bell-shape response observed over 1000-fold range

8. IP-10 Induction Over TNFa

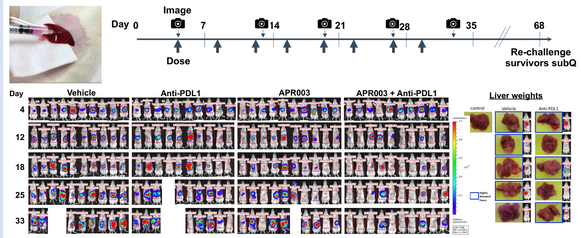


TNFa is not required for T cell mediated anti-tumor efficacy and is associated with poor tolerability at high levels (ref 4)

- APR003 induced stronger IP-10 response over TNFa compared to GS-9620 (oral TLR7 agonist, Phase 2 for HBV)
- GS-9620 caused more body weight loss at doses that produced similar IP-10 levels as APR003

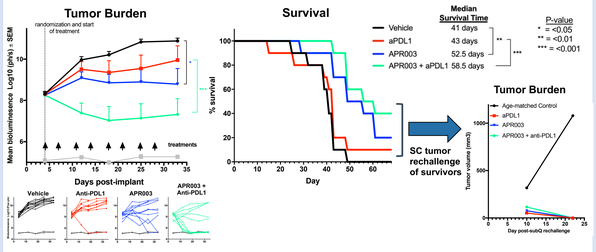
9. Orthotopic Syngeneic Liver Tumor Model

General Protocol: On Day 0, CT26-luc cells were surgically implanted into the right liver lobe of Balb/c mice. On Day 4, mice were imaged, randomized, and dosed twice-weekly for 4 weeks (APR003 and/or anti-PDL1). Tumor burden were monitored by bioluminescence imaging, and the statistical significance was calculated by 2-way ANOVA with multi-group comparison. Animal survival was tracked, and the statistical significance was calculated by log-rank test of Kaplan-Meier curves.



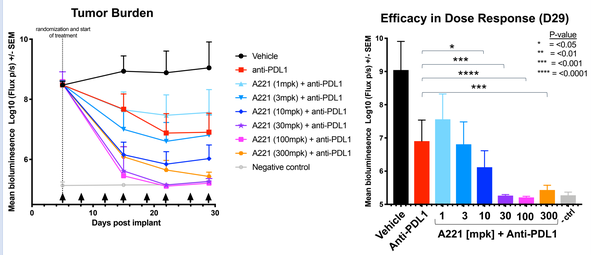
- Bioluminescence imaging allows non-invasive monitoring of tumor growth on internal organs
- Cross-validation by gross examination, liver weights, and survival

10. CT26 Liver Tumor Model



- In an orthotopic CT26 **liver** cancer model, APR003 (30 mg/kg) decreased tumor burden and increased survival as a single agent and effects were augmented in combo with anti-PDL1
- Survivors resistant to subcutaneous tumor re-challenge, suggesting adaptive systemic immunity

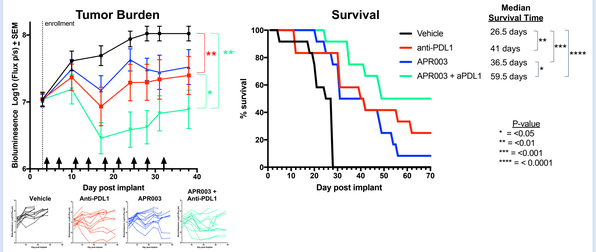
11. CT26 Liver Tumor Model (Dose Response)



- APR003 exhibited anti-tumor efficacy over a wide dose range (10 – 300 mg/kg) in combination with anti-PDL1

12. CT26 Colorectal Tumor Model

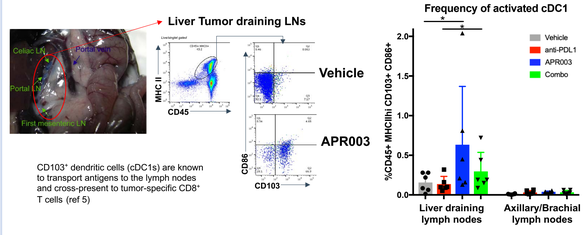
On Day 0, CT26-luc cells were surgically implanted into cecum of Balb/c mice. On Day 3, mice are imaged, randomized, and dosed twice-weekly for 4 weeks (APR003 and/or anti-PDL1).



- In an orthotopic CT26 **colon** cancer model, APR003 (30 mg/kg) decreased tumor burden and increased survival as single agent and effects were augmented in combo with anti-PDL1

13. Local Immune Priming (CD103 dendritic cells)

On Day 0, CT26-luc were surgically implanted into the right liver lobe of Balb/c mice. On Day 11, mice were imaged, randomized, and dosed on Day 11, 15, and 18, and then sacrificed on Day 19 to assess the frequency of activated CD103+ dendritic cells (cDC1) in the liver draining LNs (celiac, portal, and first mesenteric) versus non-draining LNs (axillary/brachial) by staining for MHCII and CD86.

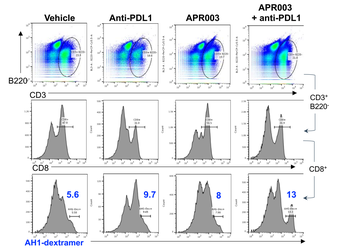


CD103⁺ dendritic cells (cDC1s) are known to transport antigens to the lymph nodes and cross-present to tumor-specific CD8⁺ T cells (ref 5)

- APR003 induced a greater frequency of activated CD103+ dendritic cells in liver draining lymph nodes, but not in distal non-draining lymph nodes

14. Increased Tumor-Infiltrating CD8 T Cells

On Day 0, CT26-luc were surgically implanted into the right liver lobe of Balb/c mice. Following 3 therapeutic doses, liver tumors were removed, and single cell suspensions were stained and analyzed by flow cytometry to assess TILs.

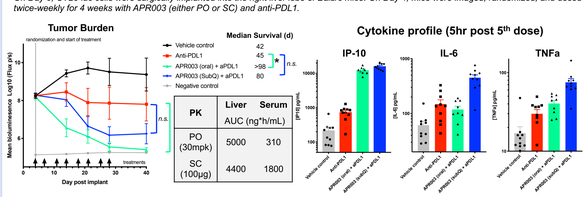


AH1 is a known antigen in the CT26 tumor cells (ref 6)

- Combination treatment of APR003 on top of anti-PD-L1 further increased tumor-specific (AH1+) CD8+ T cells in the liver tumor

15. Tissue-Specific vs Systemic Administration

On Day 0, CT26-luc cells were surgically implanted into the right liver lobe of Balb/c mice. On Day 4, mice were imaged, randomized, and dosed twice-weekly for 4 weeks with APR003 (either PO or SC) and anti-PDL1.



PK	Liver AUC (ng*h/mL)	Serum AUC (ng*h/mL)
PO (30mpk)	5000	310
SC (100μg)	4400	1800

- Systemic administration (via subcutaneous injection) of APR003 is less efficacious compared to tissue-specific oral administration at comparable liver exposure
- However, despite similar levels of IP-10 induction, systemic administration (higher serum exposure) induced more serum IL-6 and TNFa, which were not essential for anti-tumor efficacy and can lead to poor tolerability
- Therefore, GI/liver-targeted oral administration is a more optimal way to deliver TLR7 agonist to colorectal and liver cancers over systemic administration

16. Summary

- ❖ **Drug Design**: using medchem principles for liver-specific drugs (e.g. statins)
- ❖ **In vitro:** selective on TLR7 (pDC) over TLR8 (monocyte); active across species
- ❖ **PK:** pulsatile kinetics, high exposure in GI and liver, low peripheral tissue distribution
- ❖ **PD:** robust IP-10 and ISG response (desired), with minimal TNFa, IL-6, IL-10 (detrimental)
- ❖ **Efficacy:** efficacious in orthotopic models of colorectal and liver cancer (CT26, Hepa1.6, 4T1) as single agent and/or in combination with anti-PD1/L1
- ❖ **MOA:** increased activated $CD103^+$ DC frequency in DLN and tumor-specific $CD8^+$ TILs
- ❖ **Tox:** no major toxicity identified in relevant species
- ❖ **Clinical Applications:** GI malignancies with liver metastases and potentially other cancers with liver metastases
- ❖ **Product Differentiation:** potential wider therapeutic window compared to other oral TLR7 agonists or TLR7 agonist administered systemically
- ❖ **References**: 1) *Science* **2018**, *362*, 6411 ; 2) *Cancer Discov*. **2018**, *8*, 1250-1257; 3) *Curr Top Med Chem* **2013**, *13*, 857-866; 4) *Cell Reports* **2018**, *25*, 3074-3085; 5) *Trends Immunol.* **2016**, *37*, 855-865; 6) *PNAS* **1996**, *93*, 9730-9735

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