P670

Pharmacodynamic response in vitro and in vivo of novel orally administered Toll-like Receptor 7 agonists for systemic immunotherapy of cancer

James R. Appleman
Stephen E. Webber

primmune Therapeutics

ABSTRACT

Background. While ICPIs (immune checkpoint inhibitors) have fundamentally changed the practice of cancer therapy for tumors arising from many different tissues, ways to increase both response rate and durability are critically needed. Combining stimulators of innate immunity with activators of adaptive immunity should lead to better treatment outcomes. We have therefore created a series of novel orally administered, systemically acting Toll-like receptor 7 (TLR7) agonists for long-term combination therapy with enhancers of adaptive immunity like ICPIs. Our molecules incorporate a set of atypical properties critical to achieving this product profile [Appleman and Webber, 2019].

Methods. Key assays in our testing cascade include: (i) Cellular reporter assays and human PBMC assays to evaluate selectivity, potency and other characteristic aspects of cellular pharmacology (e.g. targeted cytokine and chemokine profiles) and (ii) Characterization in cynomolgus monkeys (appropriate PK with active TLR7 agonist, efficient oral delivery into systemic circulation with prodrugs of the TLR7 agonist, targeted pharmacodynamic response at relevant oral dose [gating for candidate selection]). Additional descriptive assays include: (i) Evaluation of potency and cellular pharmacology in monkey PBMCs, (ii) *In vitro* predictors of pharmacokinetics and safety, (iii) Pharmacodynamic response in rodents, and (iv) Antitumor activity in syngeneic rodent tumor models

Results. While TLR7 agonists having appropriate potency and specificity were discovered early in our program, systemic oral delivery even with an optimized prodrug approach was relatively poor. Modification of the lead TLR7 agonist itself increased efficiency of oral delivery by the prodrug from 14% for Compound PRX-B to 99% for PRX-B4 while also modestly increasing potency in PBMC assays. Surprisingly, PRX-B4 is significantly less potent in monkey plasmacytoid dendritic cells (PDCs) than in human PDCs. Consequently doses of the prodrug of PRX-B4 eliciting our targeted degree of immune induction in cynomolgus monkeys are higher than anticipated. In contrast, PRX-B3 has equivalent potency in human and monkey PBMC assays. While either of these compounds may be suitable for development, we continue to investigate additional compounds that retain the preferred features found in PRX-B3 and PRX-B4 while having greater potency in cellular assays.

Conclusions. From our original starting point - a relatively weak TLR7 agonist with no oral bioavailability - we have invented a novel series of molecules that are designed to be dosed QOD continuously over a 24-month period to appropriately engage innate immunity at a level that is well-tolerated by the patient while increasing treatment response rate and durability.

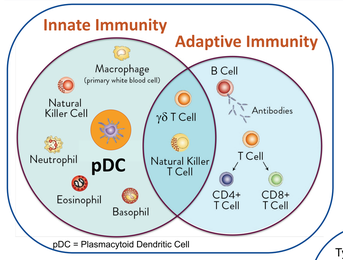
References. Appleman J, Webber, S. Discovery of a series of novel toll-like receptor 7 agonists for systemic immunotherapy of cancer [abstract]. In: Proceedings of the Annual Meeting of the American Association for Cancer Research 2019. Mar 29 - Apr 3; Atlanta, GA. Abstract # 3262.

PROJECT STATUS

➢ **PRX-B4** Meets Our Requirements for Clinical Candidate Selection

Results with additional compounds, notably PRX-B3, are also encouraging. We continue to explore agonist SAR including further investigation of the ability to tune cytokine / chemokine profile based upon TLR7 agonist structure.

BACKGROUND



Cancer immunotherapy will ultimately evolve to include systemic activation of both innate and adaptive immunity

- Cancer is a systemic disease and should be addressed as such
- The innate immune response, like the adaptive response, should be maintained at a consistent, well-tolerated level

Plasmacytoid dendritic cells (pDCs) are innate immune cells that are important in the treatment of cancer

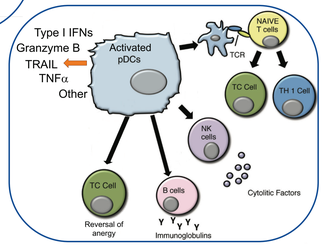
A TLR7 agonist activates pDCs, which:

- Activate tumor-fighting cells including NK and CD8+ T-cells
- Secrete soluble antitumor factors, including multi-subtype Type-I Interferons

Activated pDCs are immunostimulatory and result in a favorable response to treatment; Inactive pDCs in tumors are immunosuppressive and result in poor response to treatment

Systemic activation of pDCs increases

- immunosurveillance against micrometastases
- migration of pDCs into tumors, potentially converting cold tumors to hot tumors



Target Product Profile (TPP)

Continuous stable systemic induction of innate immunity

- Convenient QOD oral dosing regimen
- Systemic drug distribution

Safe & well-tolerated during long-term use

Compatible with immune checkpoint inhibitors

Enabled By

Selective for TLR7; not overly inflammatory (specific cytokine / chemokine profile elicited by specific subset of TLR7 agonists)

Appropriate target potency; enables "tuning" of immune induction by dose

Appropriate target engagement frequency and duration; enables sustained immune response while avoiding immune exhaustion and toxicity

Oral administration of inactive prodrug; avoids gut toxicity

TARGET CHALLENGES

Successfully navigating the path to a useful TLR7 agonist for long-term systemic therapy of cancer is not obvious

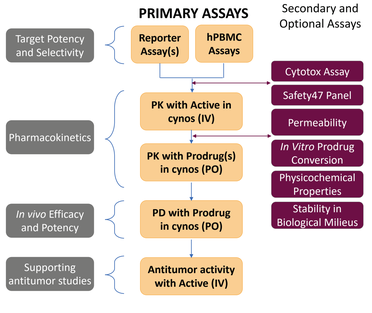
History of challenges (representative)	
Imiquimod	▪ Inability to administer systemically; highly metabolized, toxicities and lack of tolerability ▪ Not principally due to TLR7 activation
Bropirimine	▪ Cardiotoxicity (now attributed to off-target effects)
ANA975	▪ Excessive B-cell proliferation observed in repeat-dose toxicology studies in cynos ▪ Associated with TLR7 agonism in B-cell lineage
3M-852A (PF-4878691)	▪ "All or none" immune induction observed in the clinic ▪ Degree of induction not manageable by dose

Clinical studies with **ANA773** from Anadys Pharmaceuticals and **RO7020531** from Roche have demonstrated the practicality of achieving Primmune's TPP.

Drs. Appleman and Webber were instrumental in Anadys's Discovery and Translational Medicine Programs targeting TLR7 that resulted in ANA773.

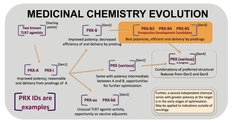
TESTING CASCADE

Abbreviated testing cascade enabled by previous discovery and clinical experience with both target and compound class



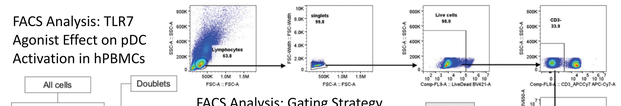
All criteria met by current lead compounds

In particular, the prodrug of **Compound B4** has met all our requirements for candidate declaration



POTENCY IN HUMAN PBMCs *IN VITRO*

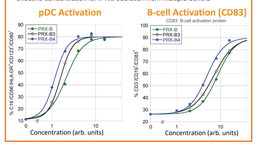
FACS Analysis: TLR7 Agonist Effect on pDC Activation in hPBMCs



FACS Analysis: Gating Strategy

ID	pDC EC_{50}	B-cell EC_{50}	Human MEC	Monkey MEC	Monkey Human
PRX-B	2.1	8.6	2.8		
PRX-B3	1.6	7.0	2.6	2.0	0.8
PRX-B4	1.2	5.0	2.0	9.0	4.5
ANA122			14.2	11.3	0.8

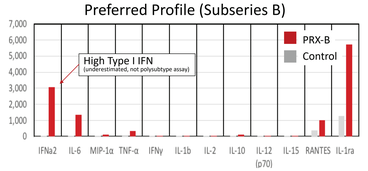
All potencies in arbitrary units; MEC is the weighted average of minimal effective concentration for IFNα secretion from multiple donors



- The greater potency of PRX-B4 in human PBMCs than in monkey PBMCs (4.5-fold) is a significant factor in projecting human clinical dose from monkey PK/PD studies.

hPBMC CYTOKINE / CHEMOKINE PROFILE

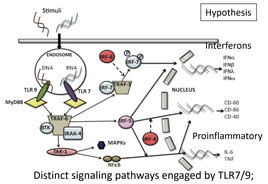
- Target profile for chronic systemic use achieved (Type I IFN-centric, modest proinflammatory cytokine production) achieved



Defining Characteristics

- MEC_{IFN} - minimal effective conc. that induces IFNα
- IFN_{max} - maximal conc. of IFNα
- Separation between conc. inducing IFN and conc. inducing proinflammatory cytokines
- Maximal conc. of proinflammatory cytokines

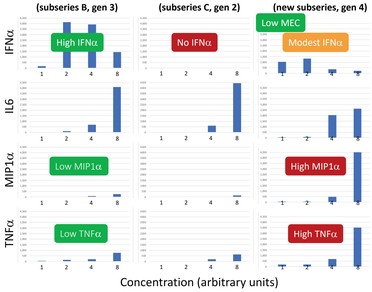
Hypothesis



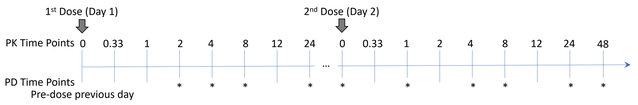
Distinct signaling pathways engaged by TLR7/9; physical separation as shown by TLR9 agonists

Diagnostic profiles of pharmacologic subseries

Data from representative compounds



PD STUDY IN CYNOMOLGUS MONKEYS



PRX-B4 administered orally as its prodrug to cynomolgus monkeys for two consecutive days at 10, 18, 48, 104, 210 mg/kg

Number animals at each dose level: 2, 2, 5 (3 receiving 2nd dose), 3 and 3, respectively

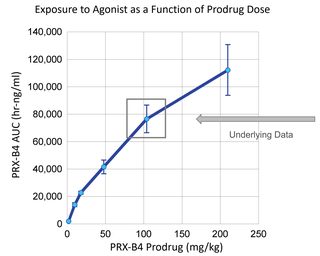
Systemic PD response assessed by selected plasma cytokine/chemokine/small analyte measures

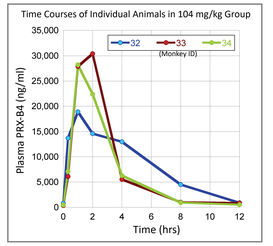
➢ PK/PD Response Supports Prodrug of **PRX-B4** as Clinical Candidate

- All dose levels well-tolerated; no adverse effects in any dose group
- Well-behaved PK across all dose groups
- Dose-dependent robust immune induction
  - Type I IFN production and typical TLR7 mediated responses
  - Minimal engagement of NFκβ proinflammatory pathway
  - Characteristic increased response on second dose administered 24 hrs after first dose
  - Variability in response consistent with that reported for the clinical-stage investigational TLR7 agonist prodrug ANA773
- Dose dependence consistent with expected PO daily human dose of 200 – 400 mg; factors considered in include calculation include:
  - Relative potencies of PRX-B4 and ANA122 (active of ANA773) in human and monkey PBMCs
  - Comparative clearance of PRX-B4 and ANA122 in monkeys
  - Clearance of ANA122 in humans

PD STUDY – DATA SUMMARY

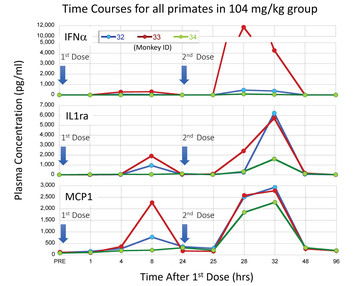
Efficient Prodrug Delivery of Active Agonist PRX-B4



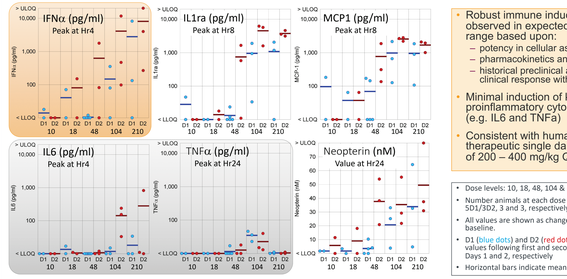


Time Dependence of Immune Induction

- Robust immune response observed in response to PRX-B4 prodrug
- Characteristic enhanced response observed on 2nd consecutive day of dosing
- No adverse effects observed in any dose group



Dose-dependent Induction Observed in Representative Immune Markers



- Robust immune induction observed in expected dose range based upon:
  - potency in cellular assays
  - pharmacokinetics and
  - historical preclinical and clinical response with ANA773
- Minimal induction of key proinflammatory cytokines (e.g. IL6 and TNFα)
- Consistent with human therapeutic single daily dose of 200 – 400 mg/kg QOD

- Dose levels: 10, 18, 48, 104 & 210 mg/kg
- Number animals at each dose level: 2, 2, 5D1/3D2, 3 and 3, respectively
- All values are shown as change from baseline.
- D1 (blue dots) and D2 (red dots) indicate values following first and second dose on Days 1 and 2, respectively
- Horizontal bars indicate mean value

ACKNOWLEDGEMENTS

Much of the laboratory experimentation reported herein was conducted at Aptuit, an Evotec Company. We would like to acknowledge the critical contributions of core project team members Filippo Andreetta, Maria Elisa Perico, Elisa Bianchini, Marisa Dal Lago, Rahul Yadav, Sadaf Kasraie and John Kamins in experimental design and interpretation, project management and alliance management.