AMP-224, a fusion protein that targets PD-1

James F Smothers, PhD

Director & Head of Immuno-Biology

Immuno-Oncology & Combinations DPU

GlaxoSmithKline Oncology R&D

TAT Congress - March 4, 2013

Disclosures and Statements

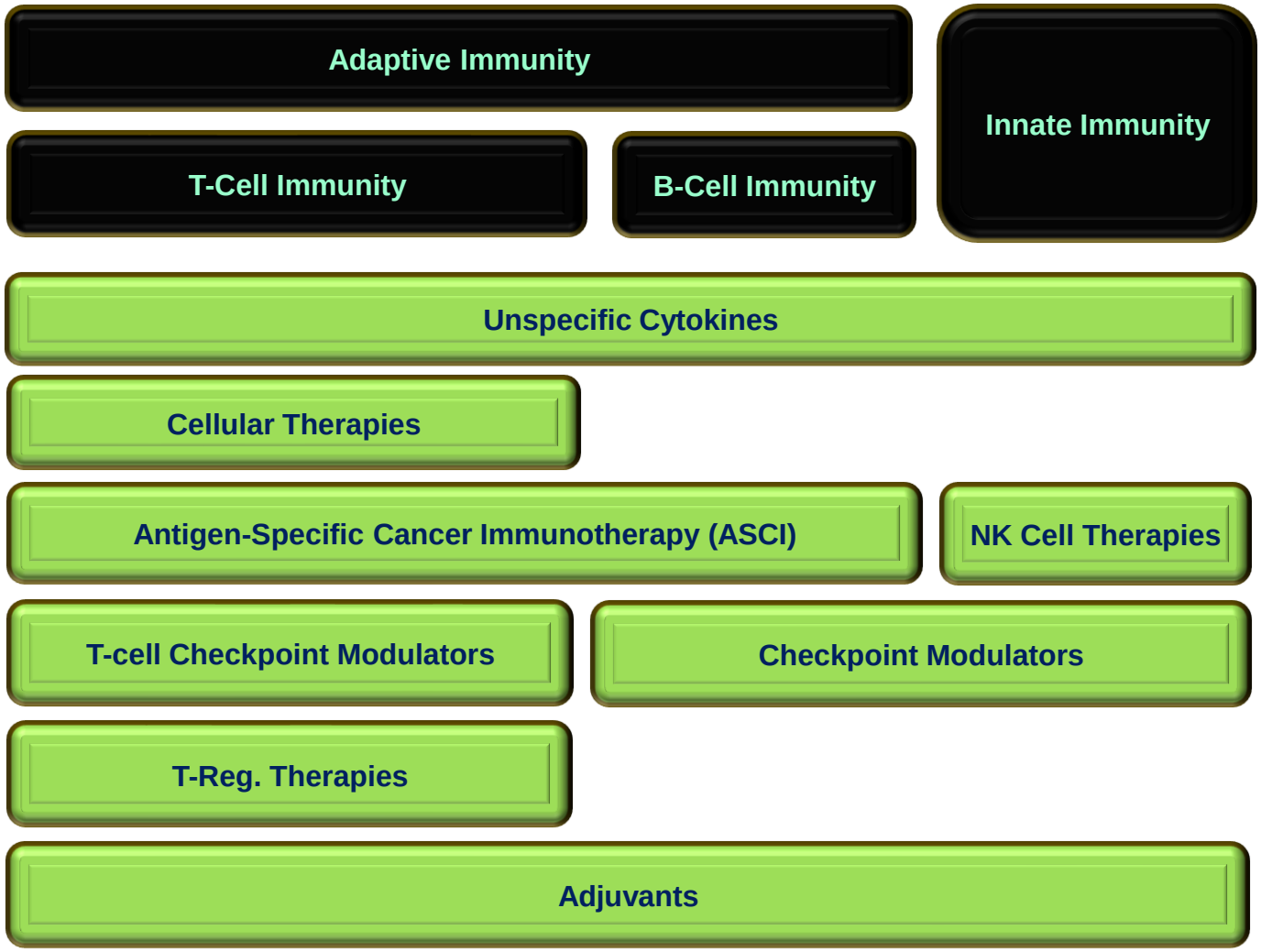
- Speaker is a GlaxoSmithKline employee and stockholder.
- Human biological samples referred to in this presentation were sourced ethically and their research use was in accord with the terms of informed consents.
- All non clinical studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed by the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.

Outline for Today's Discussion

- Immunotherapy and the PD-1 axis
- AMP-224 concept / invention
- Preclinical validation
  - Efficacy & survival in rodent tumor models
  - Differentiation from α-PD-1 mAbs
- FTIH study
  - Dose escalation & expansion schema
  - Confirmation of novel MoA in patient samples
  - Summary of exploratory biomarker plan
- Ongoing / Next Steps

GlaxoSmithKline Oncology

Immuno-Biology & Immunotherapy Strategies



GlaxoSmithKline Oncology

Targeted Strategies for Immunotherapy

- Several immunotherapy targets on T cells, APCs or tumor have been identified with varied rigor in pre-clinical validation.
- The majority of well-validated target axis opportunities are B7-CD28 or TNF / ligand family proteins.
- MoA approaches include:
  - Activation of DC (e.g., CD40, CD70, TLR)
  - Blockade of T cell inhibitory molecules (e.g., CTLA-4, PD-1)
  - Agonism of T cell co-stimulatory pathways (e.g., CD27, 4-1BB, ICOS, OX40)
  - Treg depletion or inactivation (e.g., CD25 but difficult to define selective markers to date).

Relevant reviews:

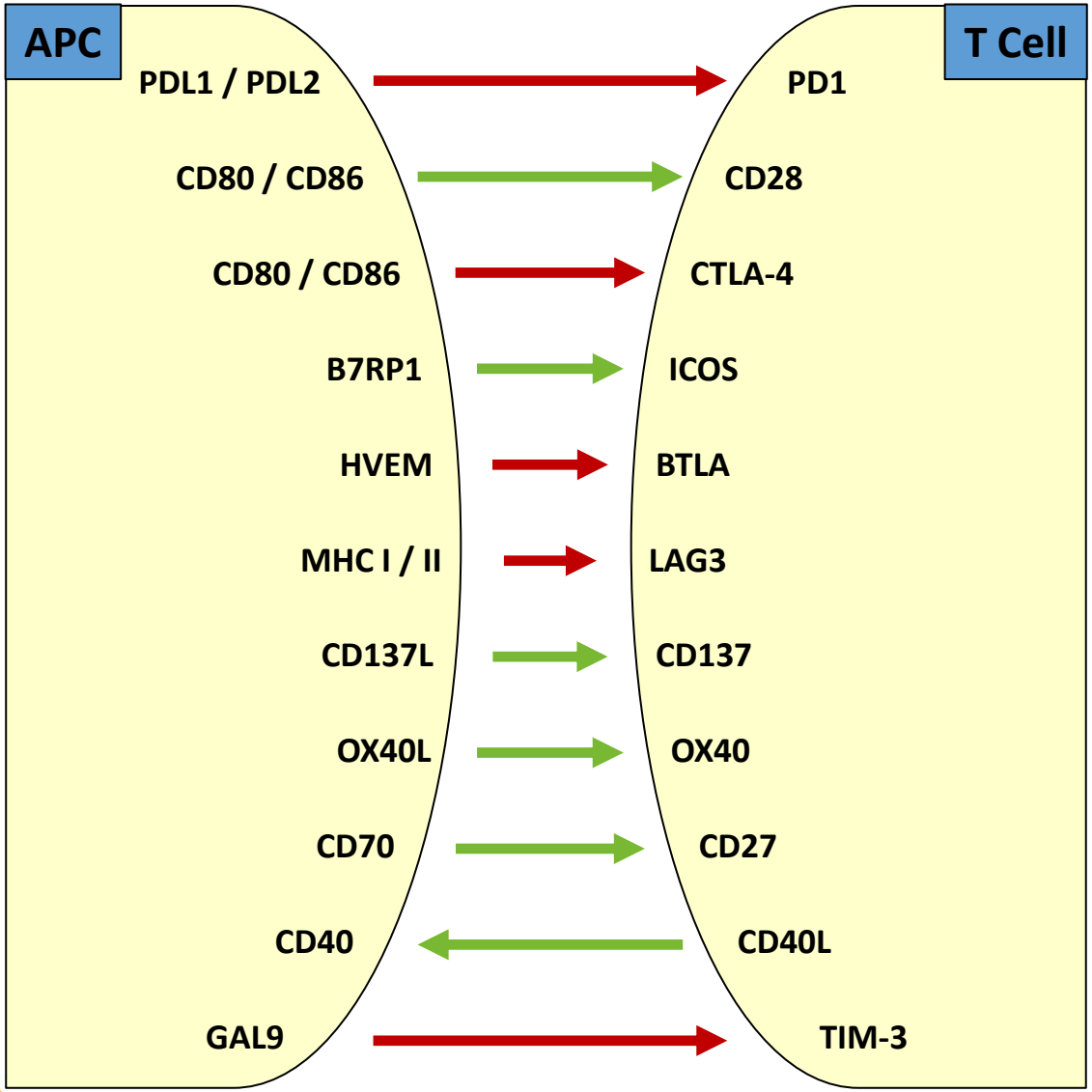
-Pardoll D *Nat Rev Cancer* 2012

-Weber *J Semin Oncol* 2010

-Peggs K et al *Immunol Rev* 2008

GlaxoSmithKline Oncology

Check Point Molecules: Targets for T Cell Modulation



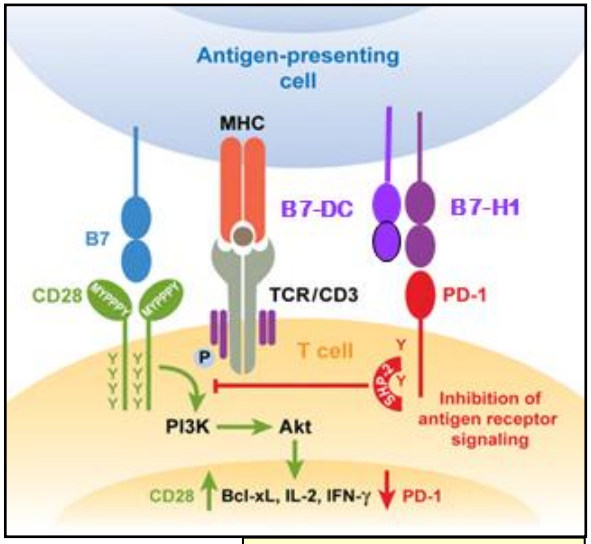
Positive signal: promotes activation / proliferation

Repressive signal: promotes suppression

PD-1/PD-L1 Targeting Agents in Clinical Trials

Compound	Company	Description	Isotype	Clinical Stage
BMS-936558	Bristol-Myers Squibb	PD-1 blocking mAb	IgG4	Phase III
CT-011	CureTech	PD-1 blocking mAb	IgG1	Phase II
MK-3475	Merck	PD-1 blocking mAb	IgG4	Phase II
RG7446	Genentech (Hoffmann-La Roche)	PD-L1 blocking mAb	Not Yet Disclosed	Phase I
BMS-936559	Bristol-Myers Squibb	PD-L1 blocking mAb	IgG4	Phase I
AMP-224	GlaxoSmithKline (from Amplimmune)	Fc-fusion of PD-L2	IgG1 Fc	Phase I

Also see http://www.clinicaltrials.gov



Keir ME et al *Annu Rev Immunol* 2008

- Differentiation amongst blocking mAbs may prove challenging as more agents enter into clinical trials.
- All clinical assets are blocking mAbs except for AMP-224.

GlaxoSmithKline Oncology

PD-1 Axis Tumor Biology and AMP-224 Composition

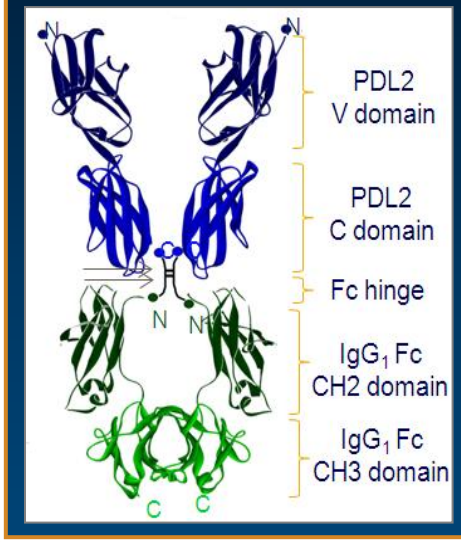
PD-1

- Up regulated on activated T cells but highly expressed on "exhausted" T cells
- Ligands are PD-L1 and PD-L2
- APC / ligand contact suppresses T-cells
- ↑ PD-1^{HI} T-cells in patient tumors

PD-L1

- Tumor expression correlates with poor survival in cancer patients*
- Expression common in solid tumors (HCC, ovarian, gastric)
- Clinical proof of concept achieved for PD-1 / PD-L1 blockade**

AMP-224



- Engineered recombinant fusion protein comprised of human PD-L2 and the Fc domain of human IgG_1
- Preferentially targets PD-1^{HI} T cells
- Enhances T cell responses (preclinical)
- Shrinks established tumors in mice
- Safe in rodent / primate toxicology studies

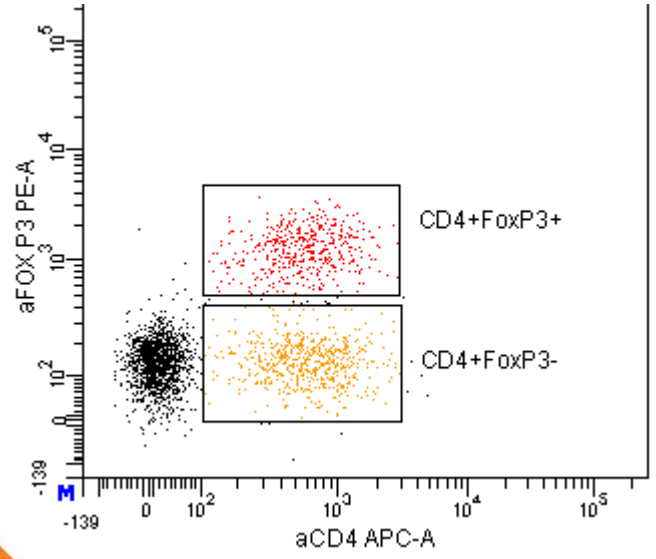
***Thompson RH et al *Cancer Res* 2006**

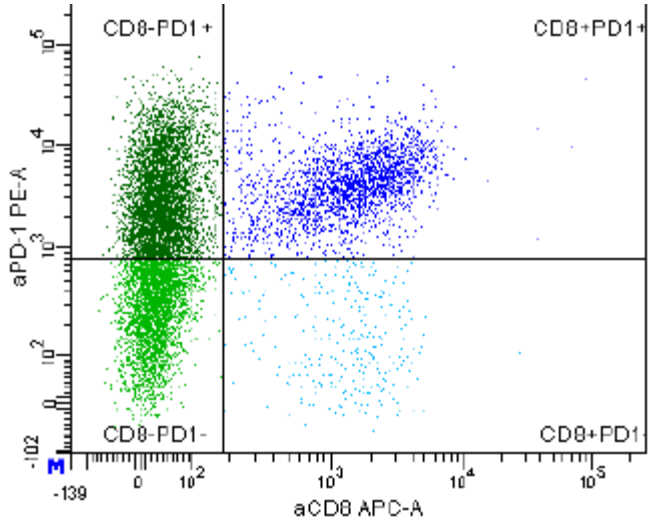
****= see Zitvogel L & Kroemer G *OncoImmunol* 2012)**

GlaxoSmithKline Oncology

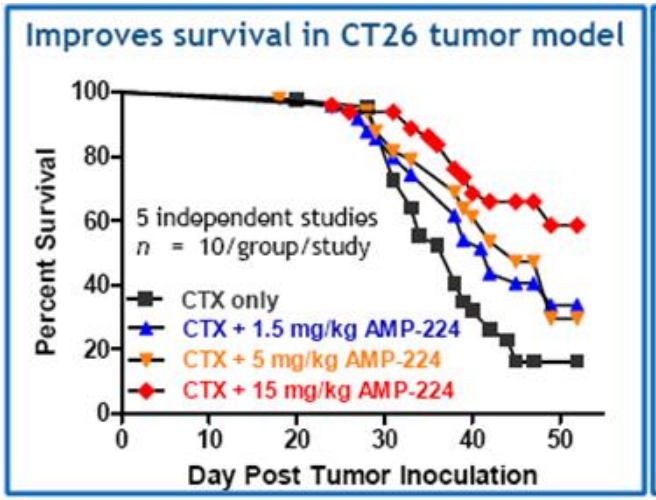
CT26 Model Recapitulates Mechanisms of Tumor Immune Evasion

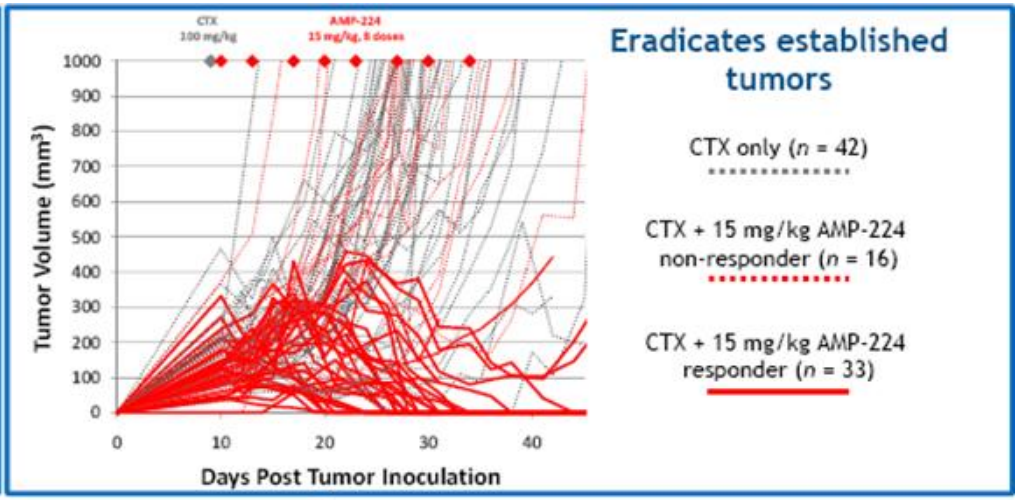
- PD-L1 is upregulated on tumor cells following implantation
  - see Sadoun *et.al.*CCR 2007
- Most CD8+ T cells in the tumor highly express PD-1 and other inhibitory markers
- About half of CD4+ T cells found within the tumor are Tregs
- Despite significant TIL infiltration, tumors grow rapidly and are 100% lethal w/o intervention

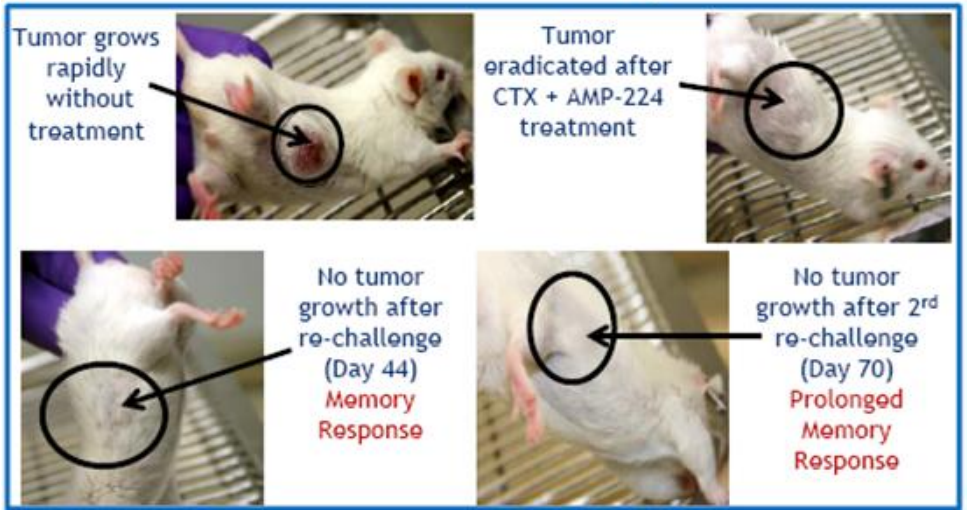


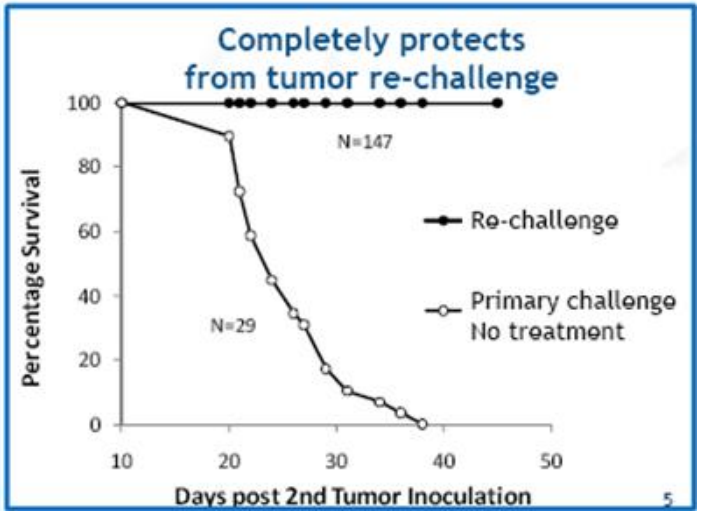


Preclinical Efficacy, Survival & Protection



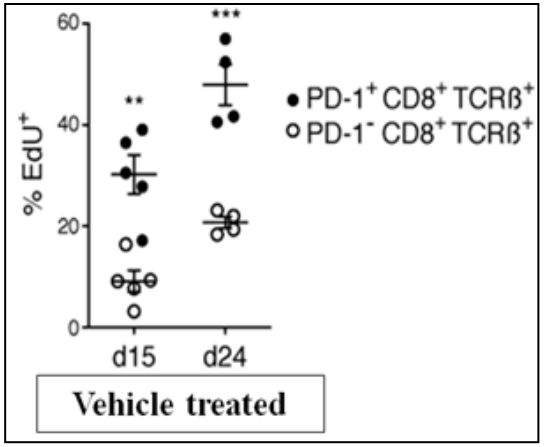


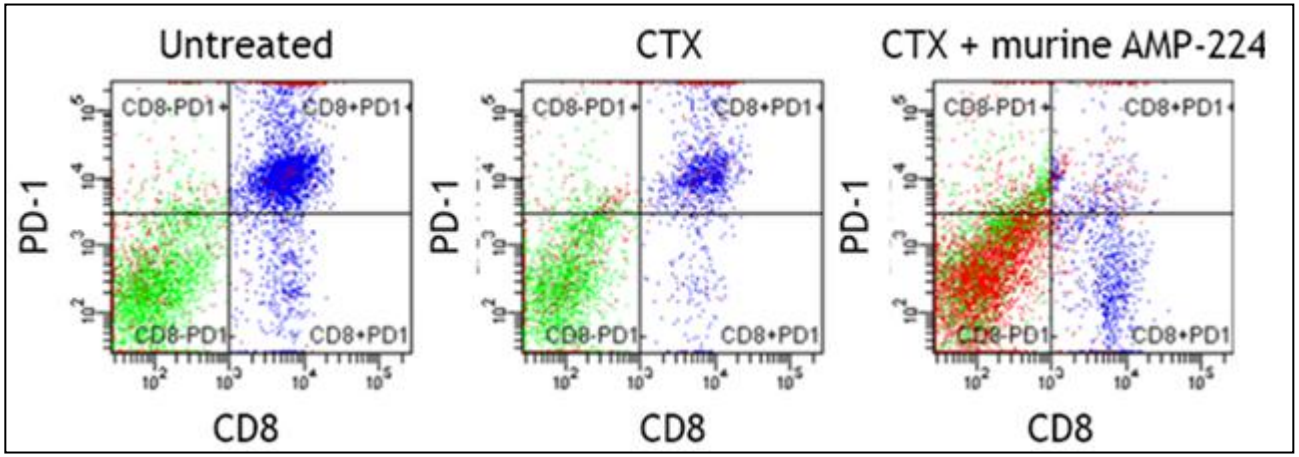


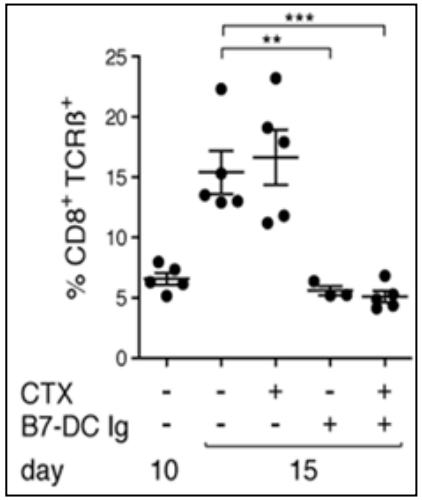


mAMP-224 Inhibits Proliferation of Dysfunctional CD8+PD-1^{HI} TILs

- Half of CD8+ PD-1^{HI} TILs are proliferating on D15 / 24
- ↓ proliferation of CD8+PD-1^{LO} TILs
- ↑ in PD-1^{HI} CD8 TILs from D10-D15 in vehicle and CTX only mice
- Hypothesis: slowly expanding PD-1^{LO} TILs have ↑ anti-tumor capacity but are out-competed by rapidly proliferating PD-1^{HI} TILs
  - mAMP224 ↓proliferation of the PD-1^{HI} TILs

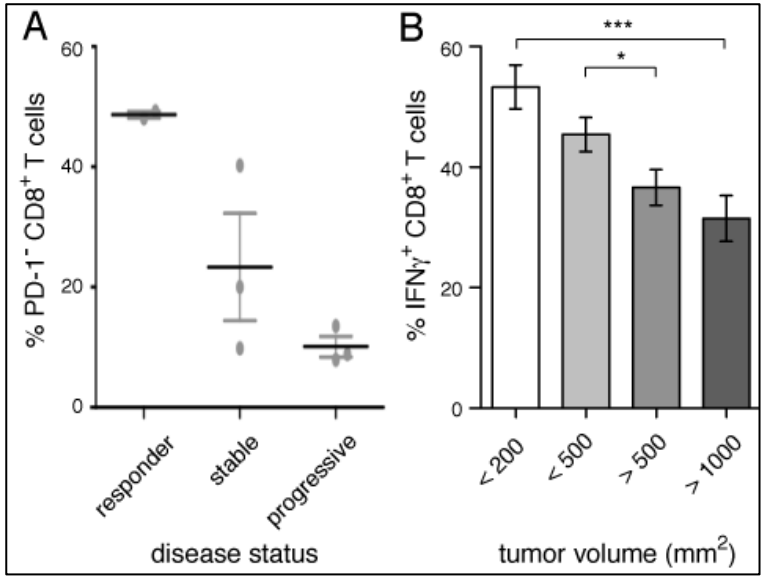






mAMP-224 Raises PD-1^{LO} TIL Counts in Responding Rodent Subjects

- CD8+PD-1^{HI} TIL population consistently low following CTX + mAMP-224
- During tumor eradication (volumes ↓ for at least 3 consecutive days) observe infiltration of CD8+PD-1- T cells
- Lower tumor burden correlates with ↑ infiltration by CD8+IFN-γ+ T cells

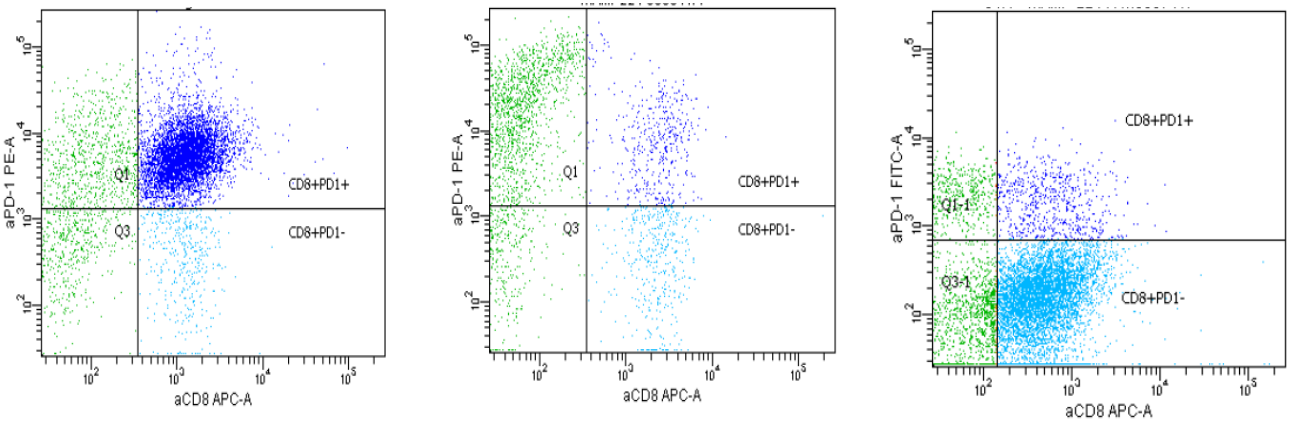


CTX + murine IgG Day 15

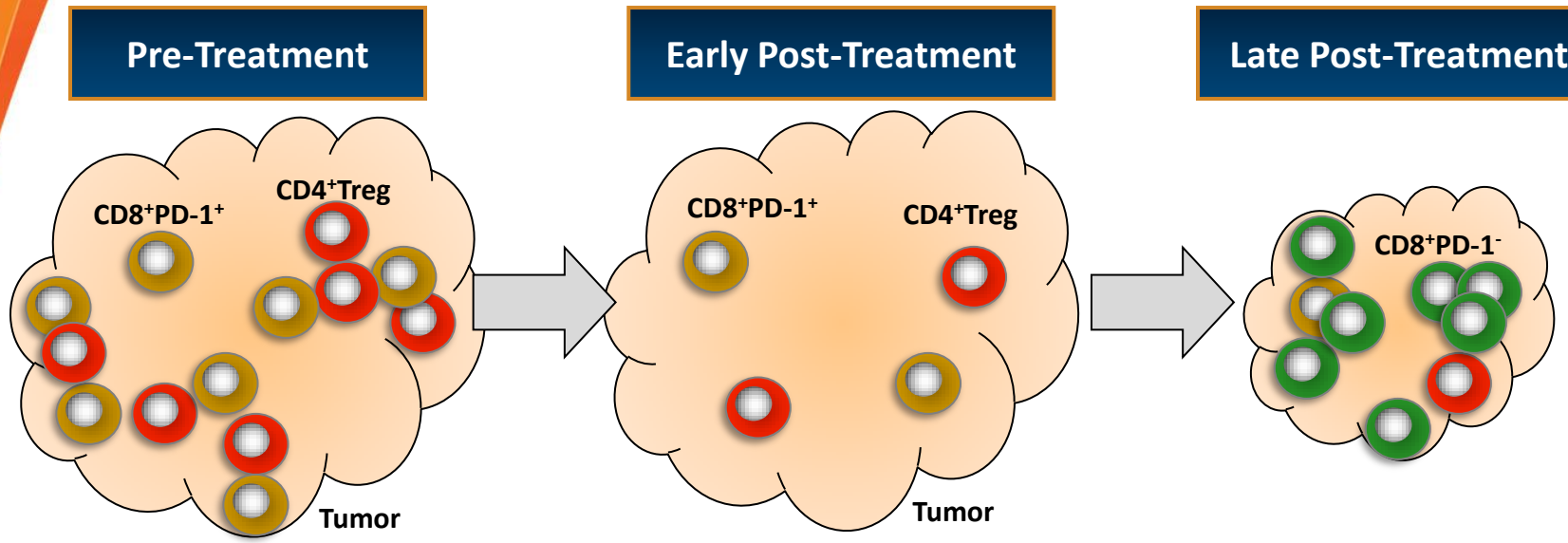
CTX + murine AMP-224 Day 15

CTX + murine AMP-224 Day 22

PD-1 Expression On CD8 T Cells



Potentially Unique MoA & Differentiation



- T cells are present in tumors and sites of chronic infection
- Some of these T cells are suppressive / dysfunctional
- $CD4^+$ Treg and $CD8^+$ $PD\text{-}1^+$ in tumor both associated with poor prognosis

- Low dose CTX and AMP-224 work together to restructure the immune environment
- Low dose CTX reduces Treg
- AMP-224 targets $CD8^+$ $PD\text{-}1^+$ HI

- A functional anti-tumor immune response emerges following treatment with low dose CTX + AMP-224
- Highly effective CD8+PD-1- cells infiltrate and kill the tumor
- T cell memory is established, conferring long-term protection

GlaxoSmithKline Oncology

Potential Combination Advantages of Novel MoA

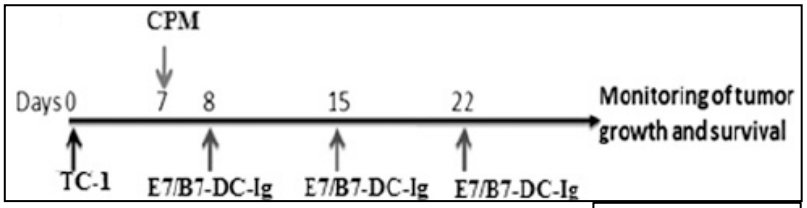


Figure 2A

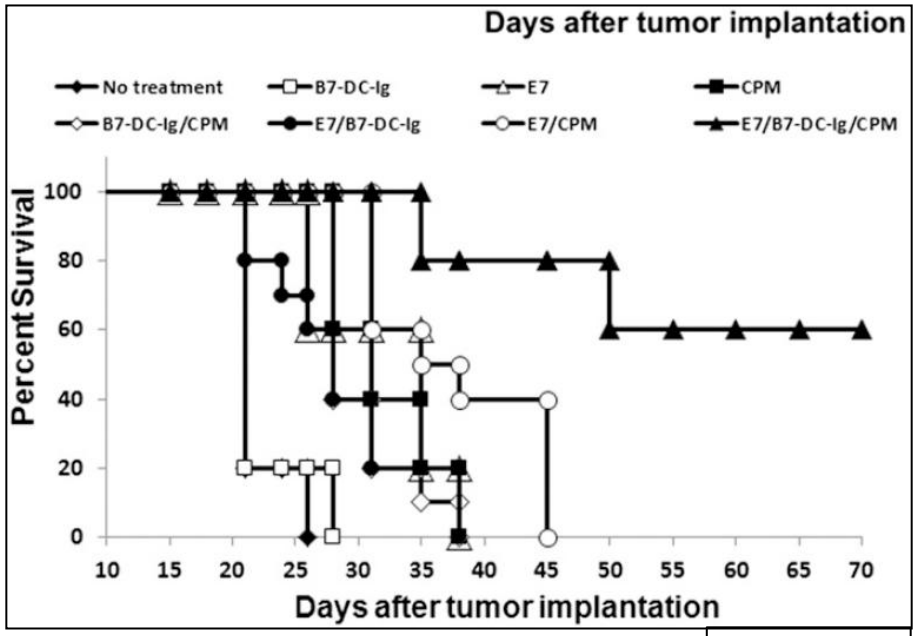


Figure 2C

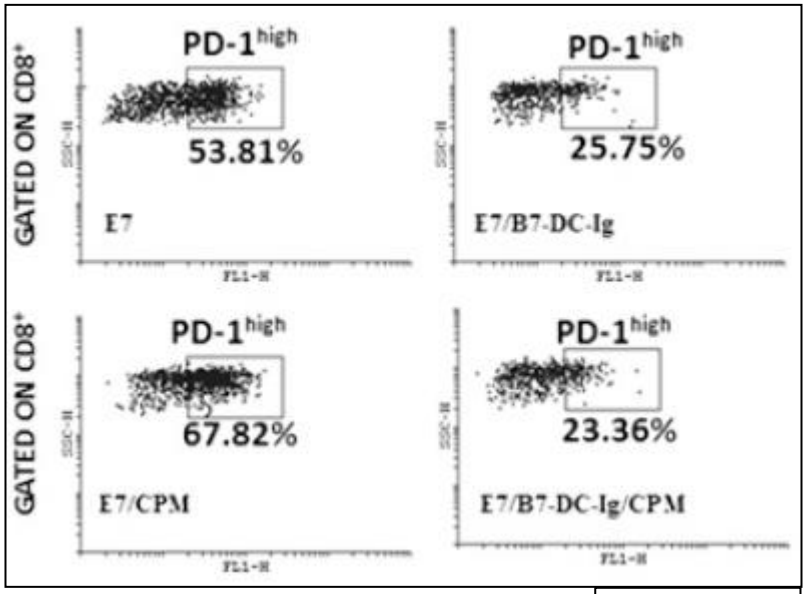


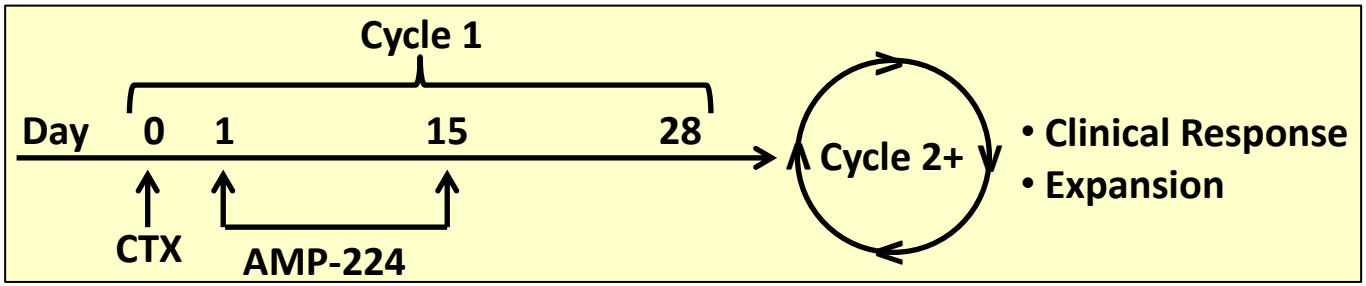
Figure 4B

M. Mkrtichyan et al. B7-DC-Ig enhances vaccine effect by a novel mechanism dependent on PD-1 expression level on T cell subsets. *Journal of Immunology.* 2012

Summary of Preclinical Rodent Model Efficacy / Survival Benefit

Model	Origin	Protocol (timing of admin)	Outcome
P815 SC	Mastocytoma	Murine AMP-224 only (D6 Tumor)	>40% Tumor Eradication / Long Term Survival
EL4 SC	Lymphoma	Murine AMP-224 only (D1 Tumor)	100% Tumor Growth Prevention
CT26 SC	Colorectal	Murine AMP-224 ±Cytoxan (D9 / 10 Tumor)	>70% Tumor Eradication / Long Term Survival
SP1 Lung Met	Prostate Metastasis	Murine AMP-224 + Cytoxan (D4 Tumor)	~50% Overall Survival
CT26 Hemispleen Liver Met	Colorectal liver metastasis	Murine AMP-224 + Cytoxan + Listeria AH1 (D10 Tumor)	10-50% Overall Survival
B16 SC	Melanoma	Murine AMP-224 + GVAX (D3 Tumor)	90% Overall Survival with 500 ug on D80
TC-1 tumor model SC	Lung (epithelial)	Murine AMP-224 + Cytoxan + HPV16 E7 Ag vaccine (3-4 mm Tumor)	~60% Overall Survival

Phase 1 / FTIH Trial Overview

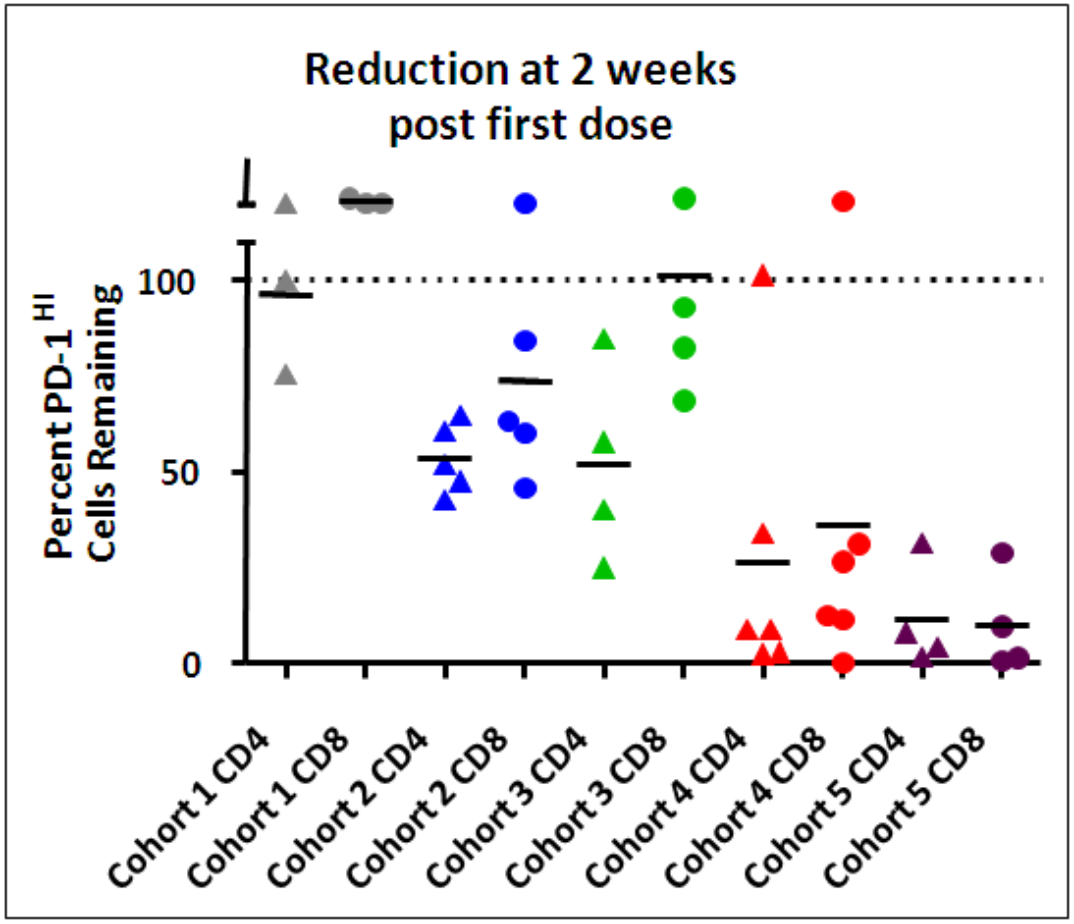


- Open-label, multi-center two stage study
  - Regimen = CTX (Day 0) & AMP-224 (Day 1 & 15) of each 28 day cycle
  - Evaluate safety, tolerability, PK & PD
- Accelerated titration until Grade 2 AE, then 3+3 design
- 2 stages: Dose-escalation and Expansion
  - Dose-escalation: 26 patients
    - Standard 28 day treatment regimen (CTX Day 0, AMP-224 Days 1 & 15)
    - 0.3, 1, 3, 10, & 30 mg/kg dose levels
  - Expansion Phase: 18 patients
    - Standard 28 day treatment regimen (CTX Day 0, AMP-224 Days 1 & 15)
    - 10 or 30 mg/kg dose levels

AMP-224 Evinces Dose-Dependant Reduction in Circulating PD-1^{HI} Cells

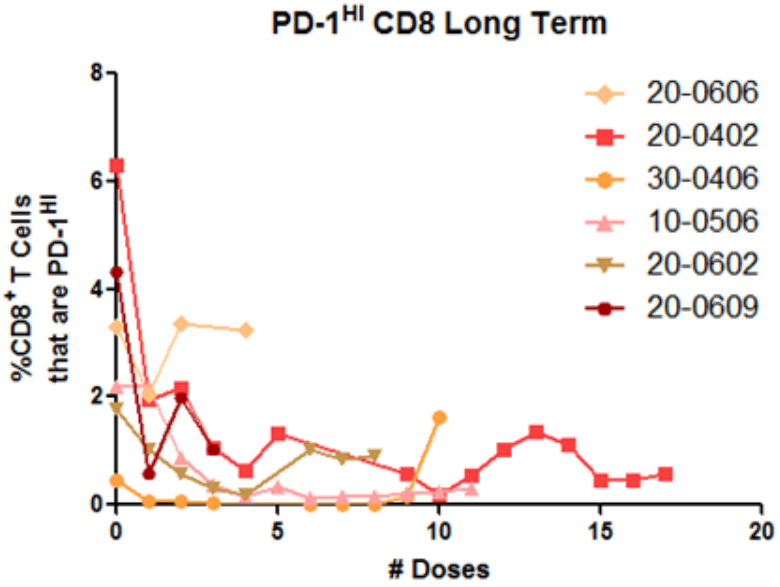
Cohort	Dose (mg/kg)
1	0.3
2	1
3	3
4	10
5	30

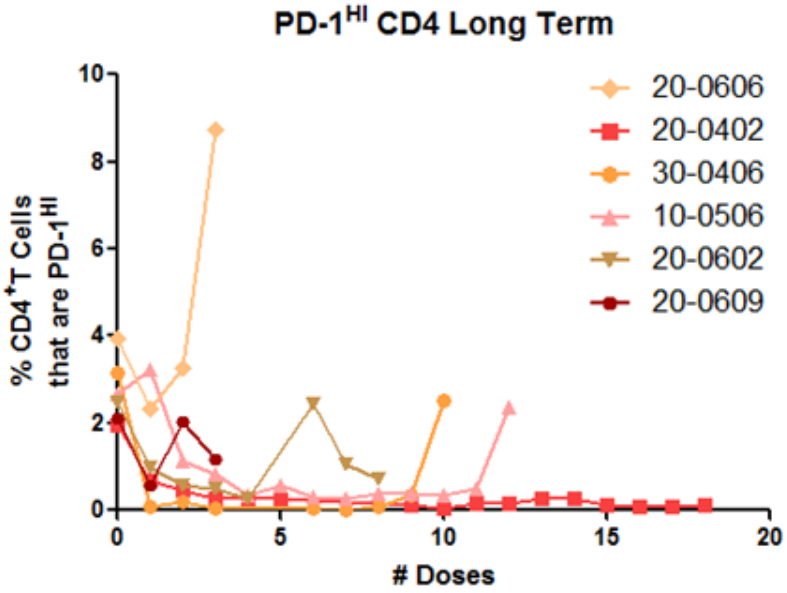
- Affects both CD4 and CD8 populations
- Range overlapping between 10 and 30 mg/kg cohorts
- Agreement with preclinical MoA observations in TILs



GlaxoSmithKline Oncology

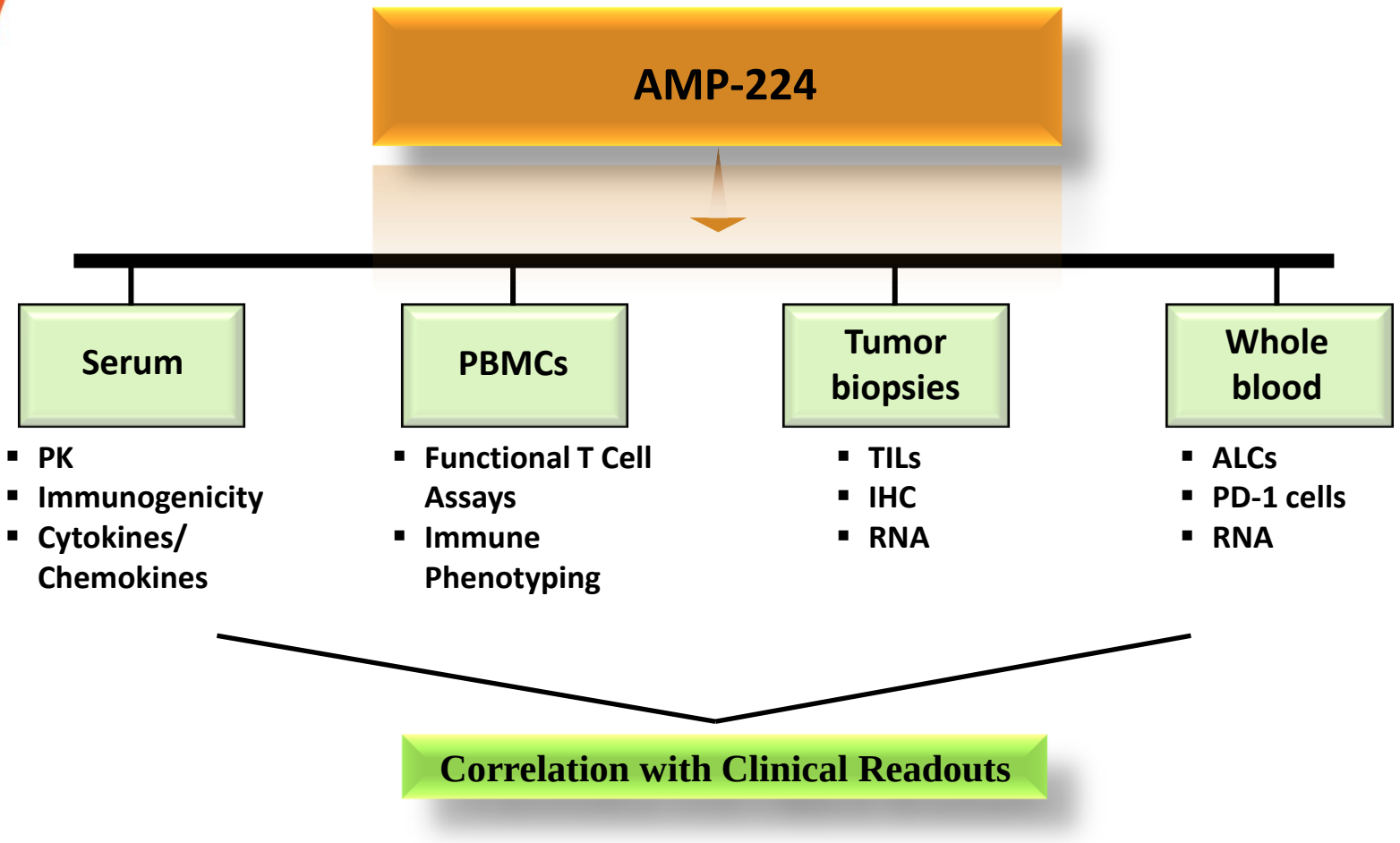
Sustained Reduction in PD-1^{HI} Cells Observed at 10 & 30 mg/kg





- Data from select patients in cohorts receiving either 10 or 30 mg/kg
- PD-1^{HI} CD8 cells are reduced but not completely eliminated in most patients
- PD-1^{HI} cells rebound after treatment ends in pts 0406 (WK12) and 0506 (D56)
- Patients who progress quickly do not evince reduced PD-1^{HI} cells (pt 0606)

Biomarker Plan: Exploring Predictive & Pharmacodynamic Value



Ongoing / Next Steps - I

- Complete expansion phase of FTIH trial
- Determine changes in PD-1 / PD-L1 levels in paired patient samples and compare TILs vs. peripheral T cells
  - IHC (tumor biopsies)
  - Flow cytometry (PBMCs)
- Determine expression levels of biomarkers in paired patient samples
  - Define pharmacodynamics of T cell indicators of exhaustion / suppression vs. activation / effector functionality
    - Flow cytometry (PBMCs)
    - bDNA analysis (PBMCs and tumor biopsies)

Ongoing / Next Steps - II

- Complete internal and collaborative studies to inform on value and composition of future clinical evaluation:
  - Translational / MoA studies of patient samples from other cancer trials
    - IHC of exploratory biomarkers followed in AMP-224 phase I
    - AMP-224 interaction with TILs (e.g., from melanoma patients)
  - Preclinical combination studies with:
    - Immunogenic tumor-targeted / debulking agents
    - Other immunotherapies (e.g., additional T cell checkpoint modulators)

Acknowledgements

Sara Brett

Mohammed Dar*

Axel Hoos

David Figueroa

Fiona Germaschewski

Sol Langermann

Shannon Marshall

Rena May

Margaret Fleming

*** = former GSK employee**

gsk GlaxoSmithKline Oncology