



IMO-4200, a Novel TLR7 and TLR8 Dual Agonist, Enhances Antitumor Effect of Ofatumumab, Rituximab and Cytotoxics in Preclinical Models of Hematological Malignancies

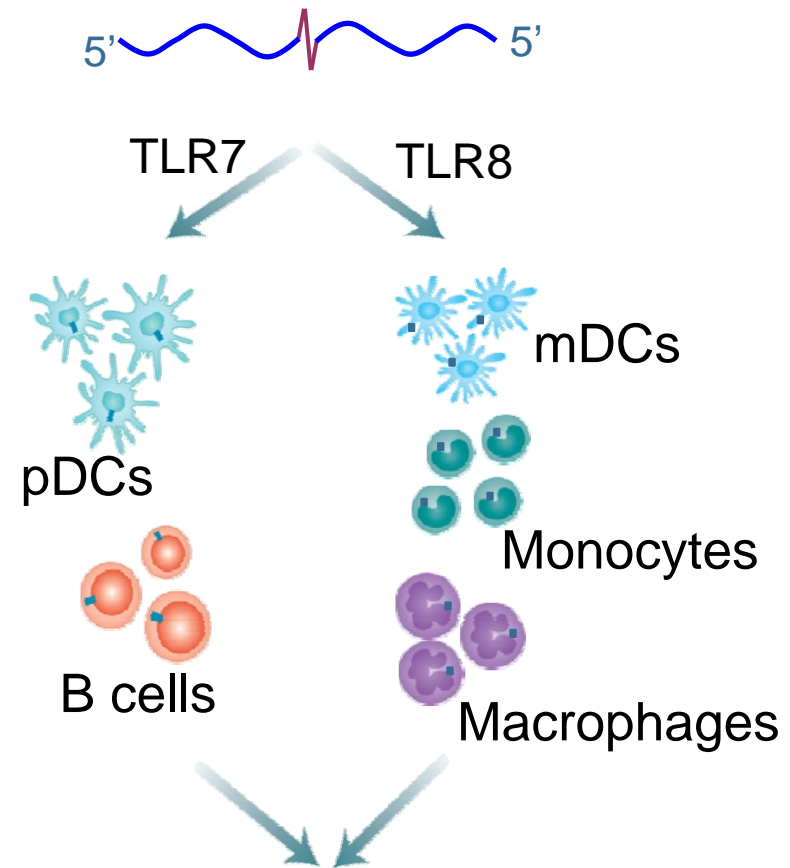
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Abstract number 3724

IMO-4200: Dual Agonist Targets TLR7 and TLR8

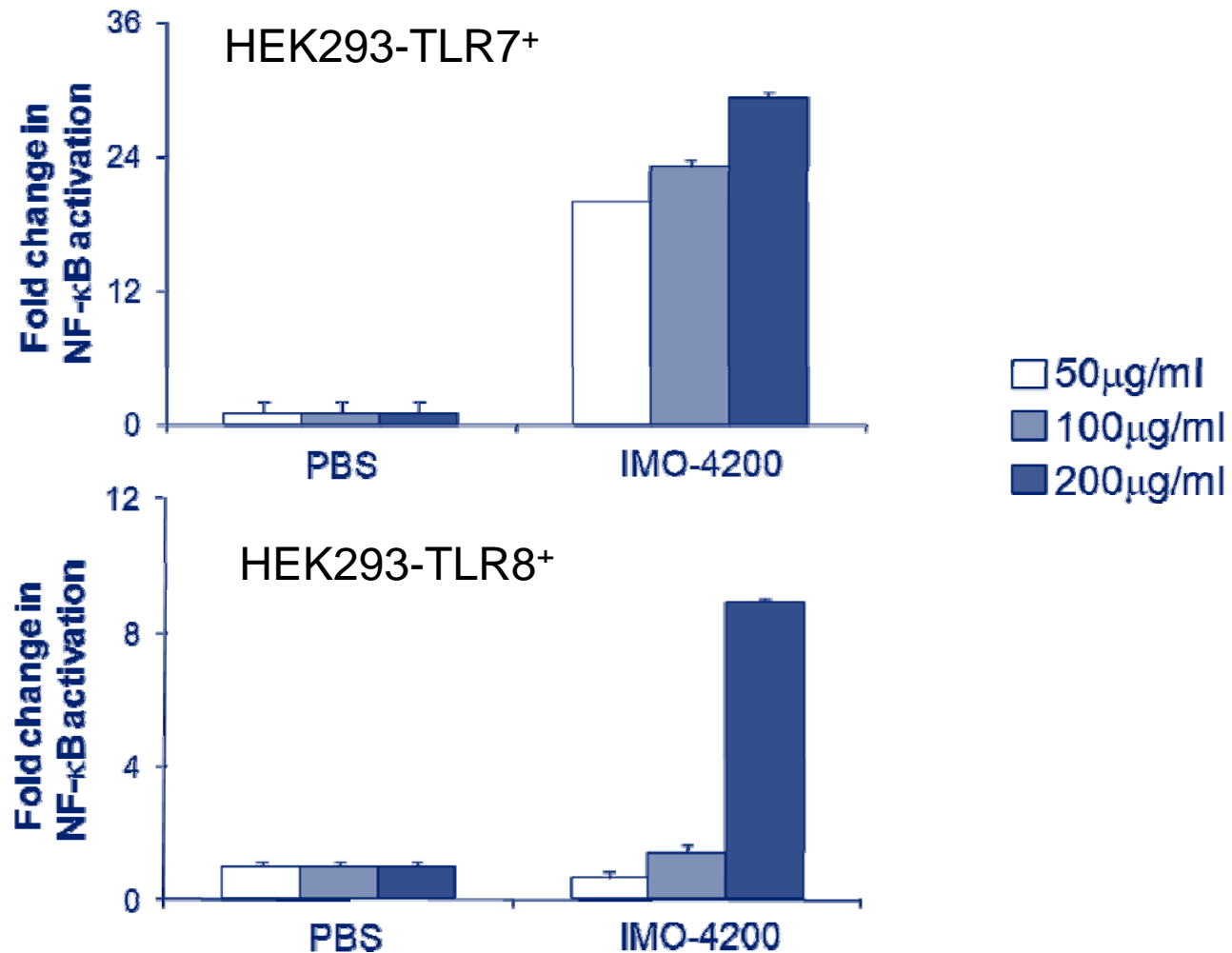
- RNA based
- Proprietary synthetic structures
- Metabolically stable
- Induces immune responses in non-human primates following s.c. administration
- Exerts antitumor effects in solid tumor xenograft models
- Identified IMO-4200 as a lead candidate for clinical development



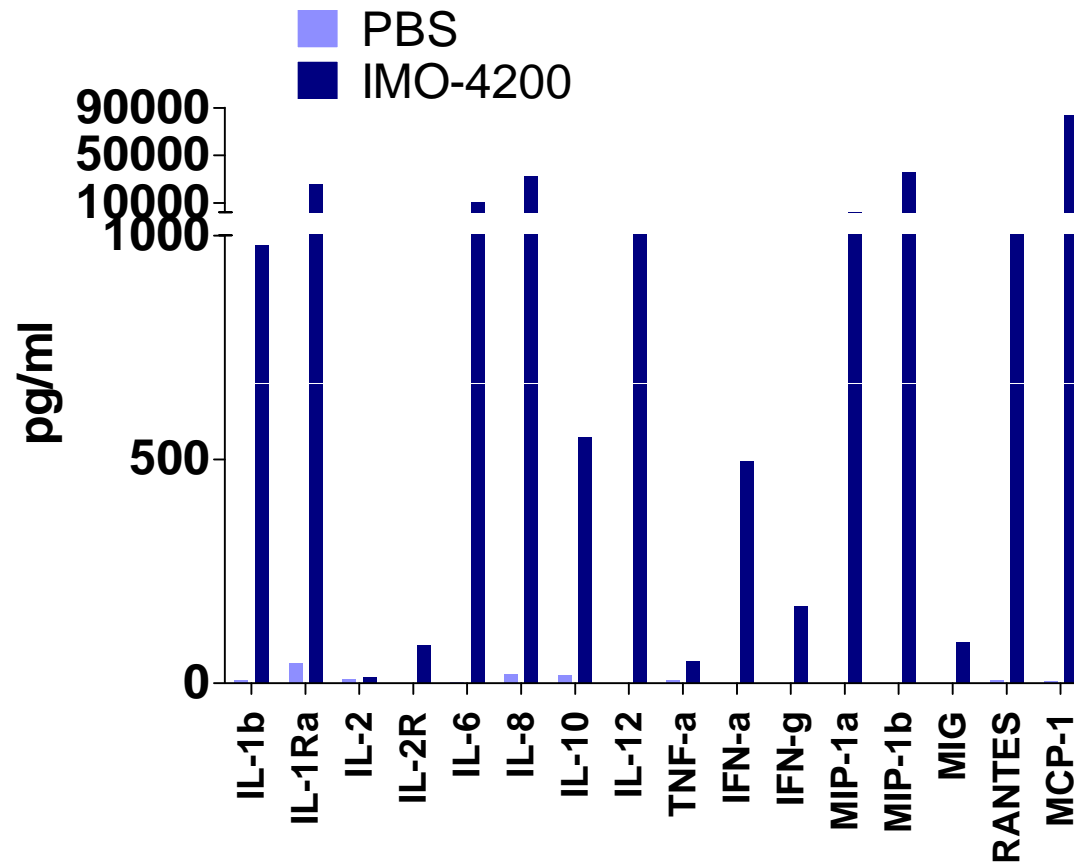
**Expression of Th1 cytokines
Immune activation**

pDCs = plasmacytoid Dendritic Cells
mDCs = myeloid Dendritic Cells

IMO-4200 Activates TLR7 and TLR8

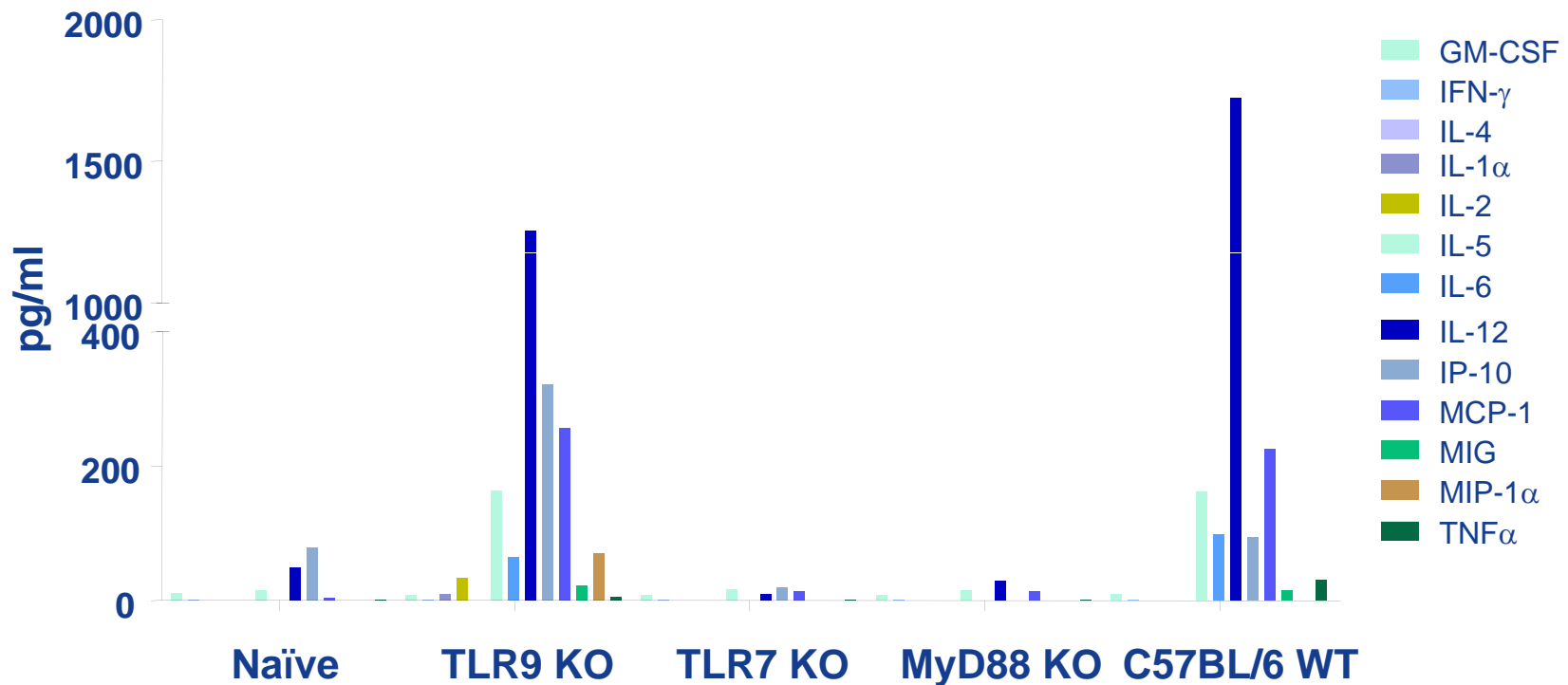


IMO-4200 Induces Cytokine/Chemokine Production in Human PBMC



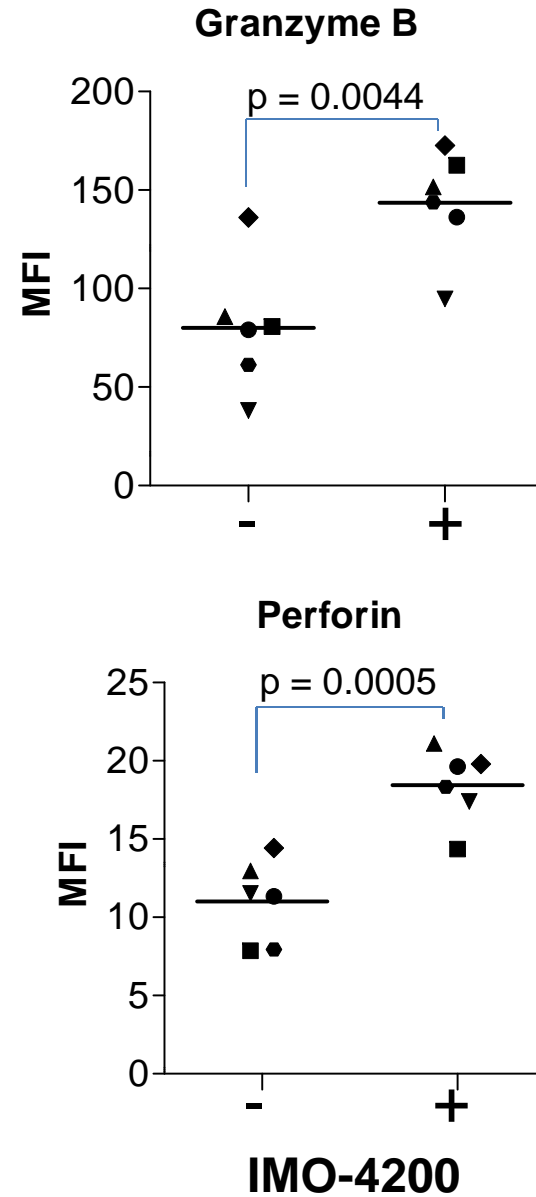
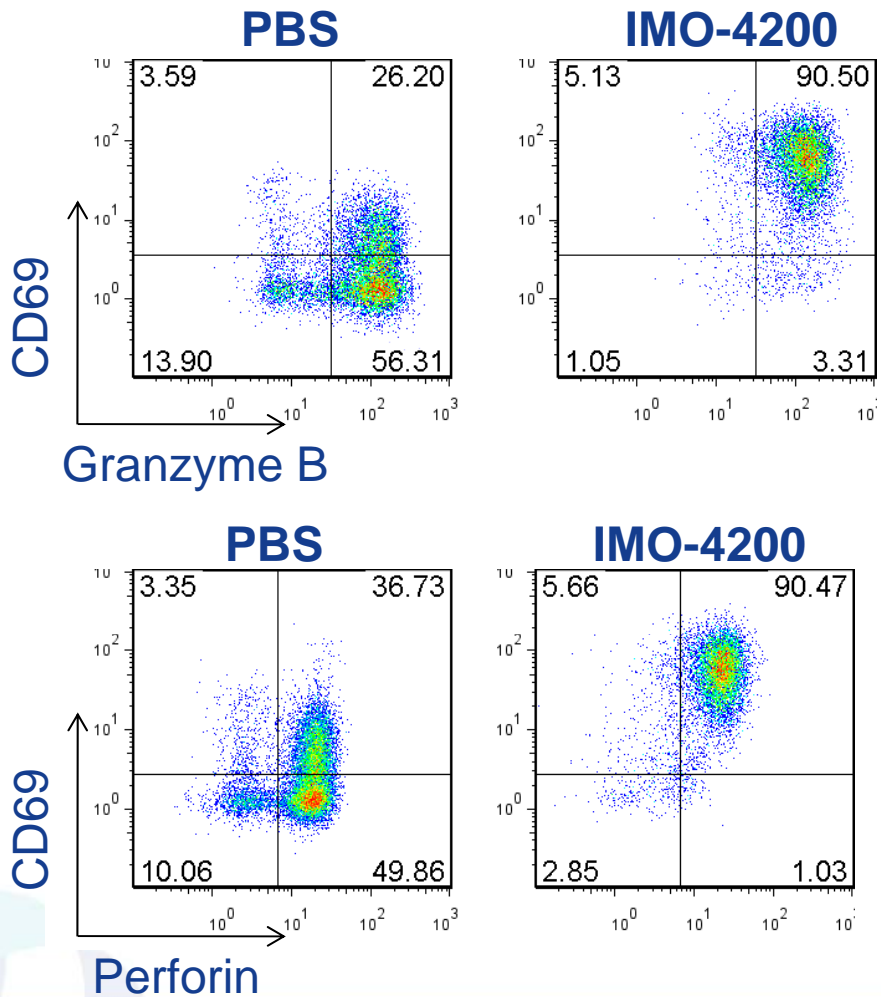
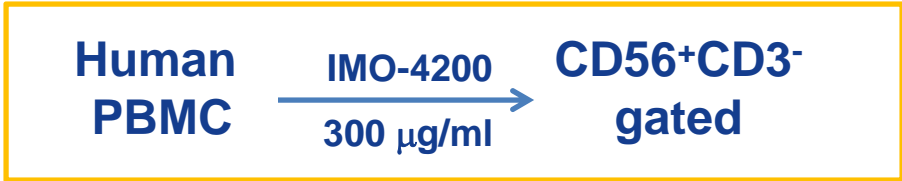
PBMC + IMO-4200 $\xrightarrow{300 \mu\text{g/ml}}$ Cytokine/chemokine assay

IMO-4200 Activates Immune System via TLR7-Mediated MyD88-Dependent Signaling Pathway

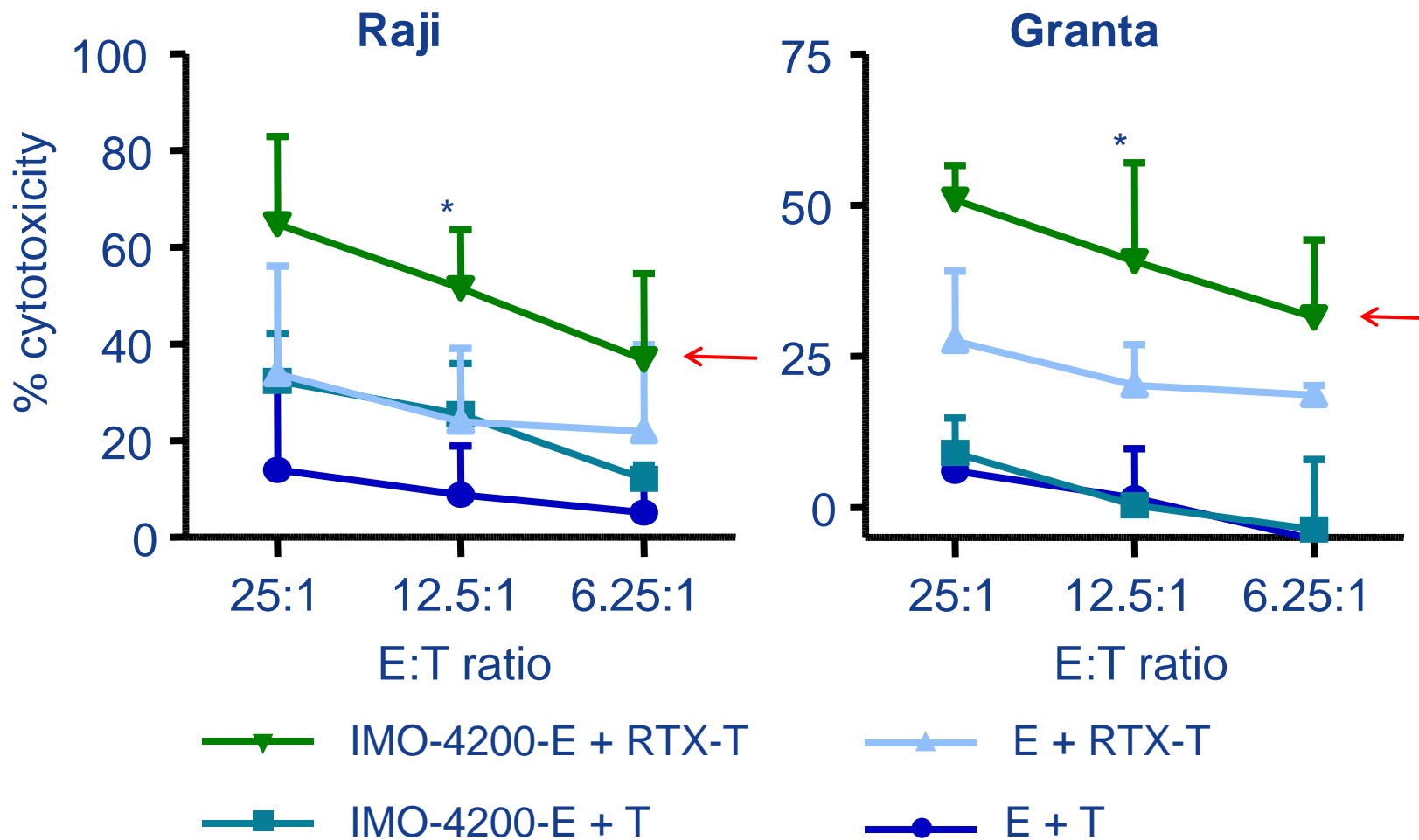


Blood samples were collected 2 hr after injection of IMO-4200 (50mg/kg) and serum samples were analyzed by multiplex assays with Luminex xMAP system.

IMO-4200 Increases Frequency of Activated NK Cells

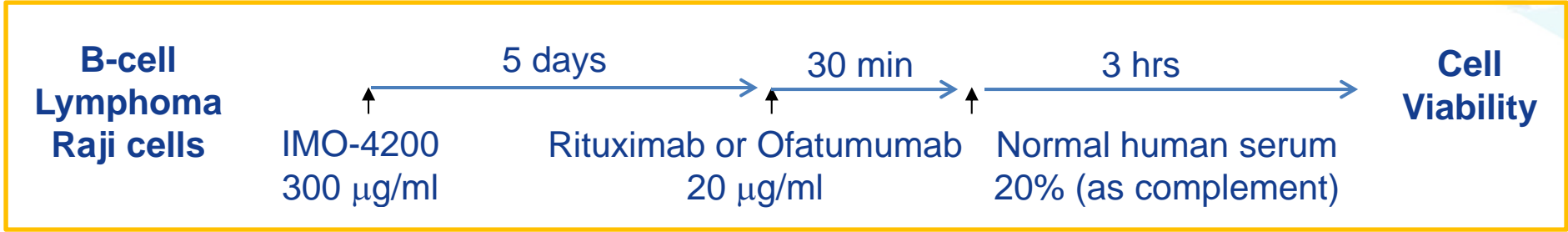


IMO-4200 Increases Rituximab-Mediated ADCC

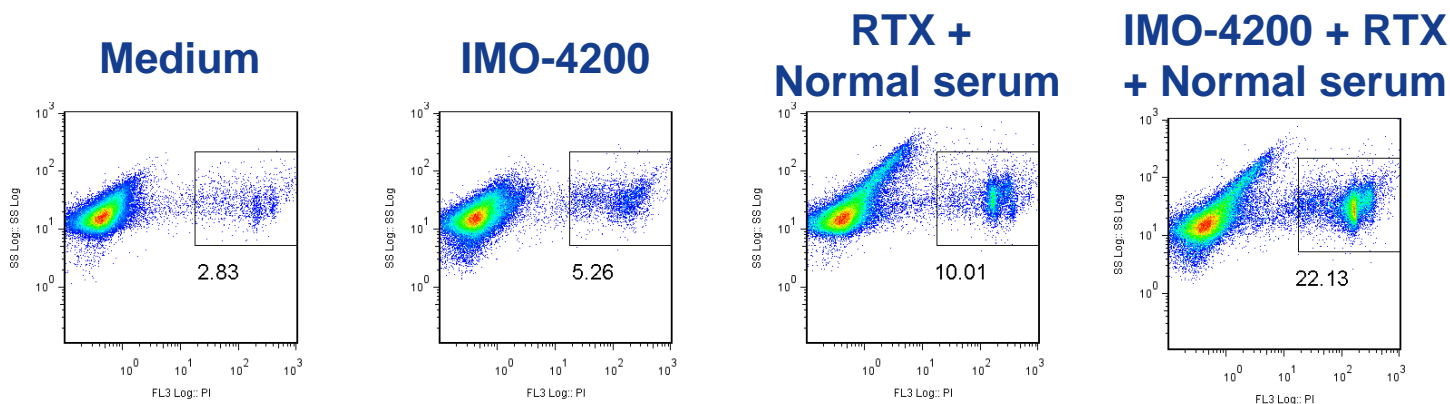


* $p \leq 0.05$ IMO-4200-E + Ritux-T vs E + Ritux-T

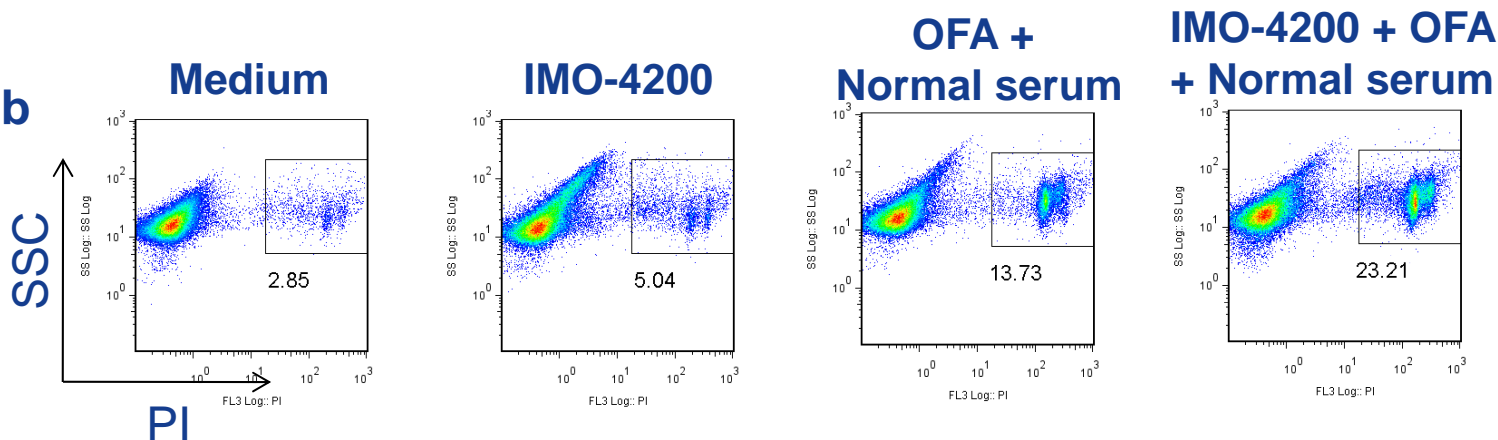
IMO-4200 Sensitizes Both Anti-CD20 mAb-Mediated CDC



Rituximab (RTX)



Ofatumumab (OFA)



Enhancement of mAb-mediated CDC by IMO-4200 is TLR7-Dependent and is Associated with Up-regulation of CD20 and Activation Markers



TLR7 Expression

Ramos
(B-cell lymphoma)

+

Raji
(B-cell lymphoma)

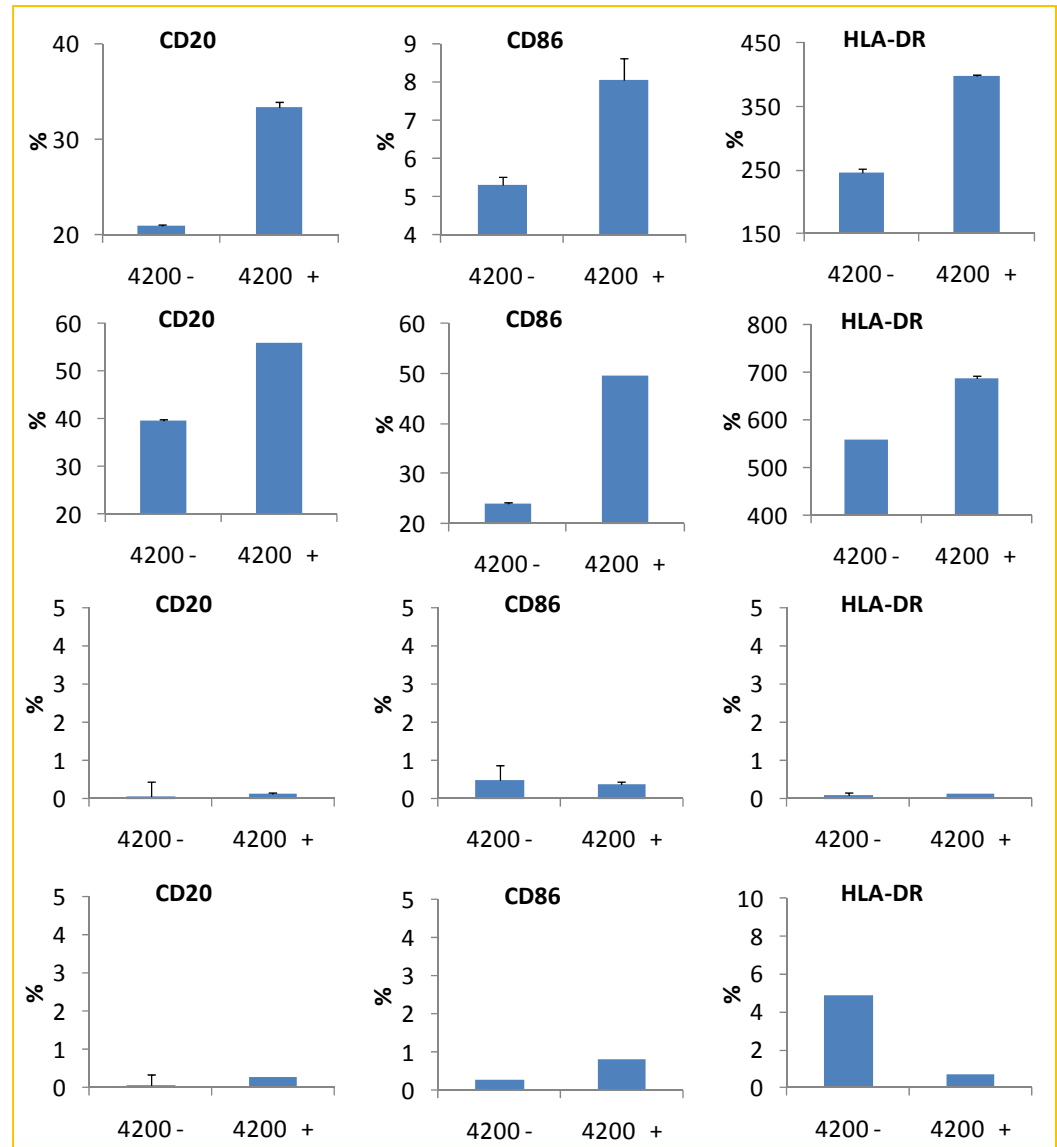
+

HSB-2
(T-lymphoblastic leukemia)

-

K562
(Myelogenous leukemia)

-



B-cell Malignancies Display Functional TLR7 as Normal B-cells

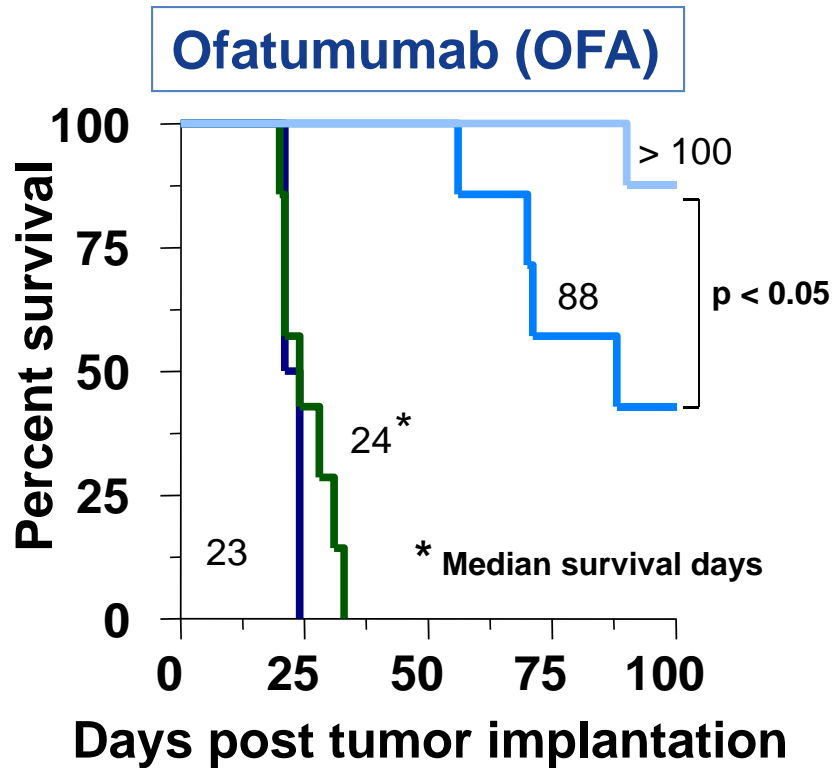
Cell line	TLR7 mRNA (AU*)	Cytokine/Chemokine Induction (pg/ml)			
		MIP-1 α		MIP-1 β	
		Medium	IMO-4200	Medium	IMO-4200
Raji	1.1 x 10 ⁻⁴	295.4	476.9	215.8	268.4
HBL-2	2.1 x 10 ⁻³	130.1	237.7	220.2	365.7
Namalwa	2.8 x 10 ⁻²	0	709.3	12615	12674
RSV;11	5.2 x 10 ⁻³	0	647.3	0	3714.7
K562	0	18.8	2	14.7	0
HSB-2	0	4.1	2	6.9	0

Only TLR7 positive tumor cells responded to the treatment of the IMO-4200 by increasing secretion of MIP-1a and MIP-1b, suggesting that TLR7 in these B-cell malignant cells is functional.

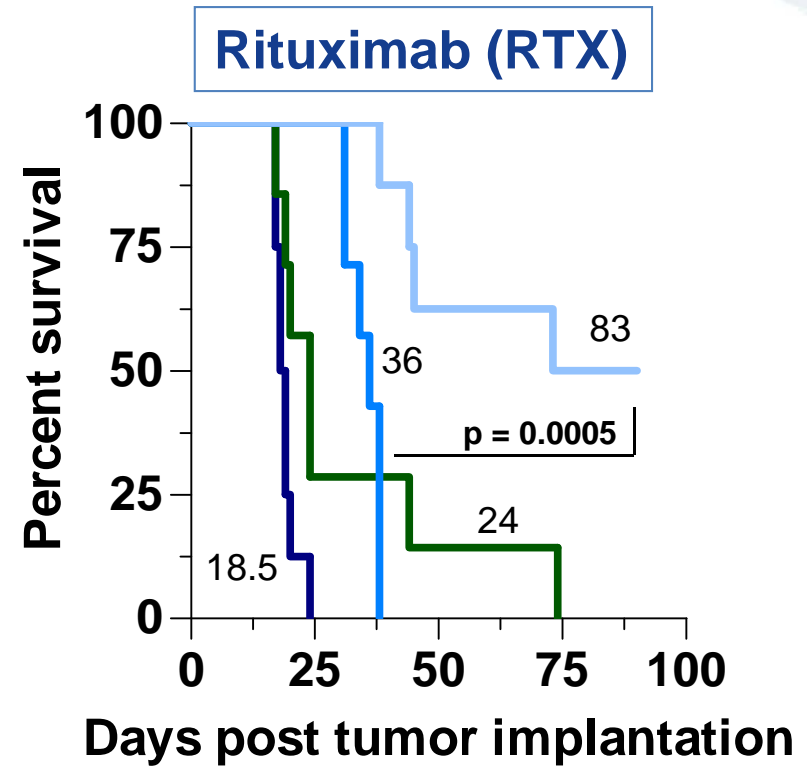
*The expression of TLR7 was analyzed by real-time semi-quantitative RT-PCR, and was expressed in arbitrary units normalized levels of hTLR7 mRNA to the endogenous control GAPDH.

Cells + IMO-4200 $\xrightarrow{300 \mu\text{g/ml}}$ Cytokine/chemokine assay

Synergistic Effects of IMO-4200 on mAb-Based Therapy – Raji Xenograft Model



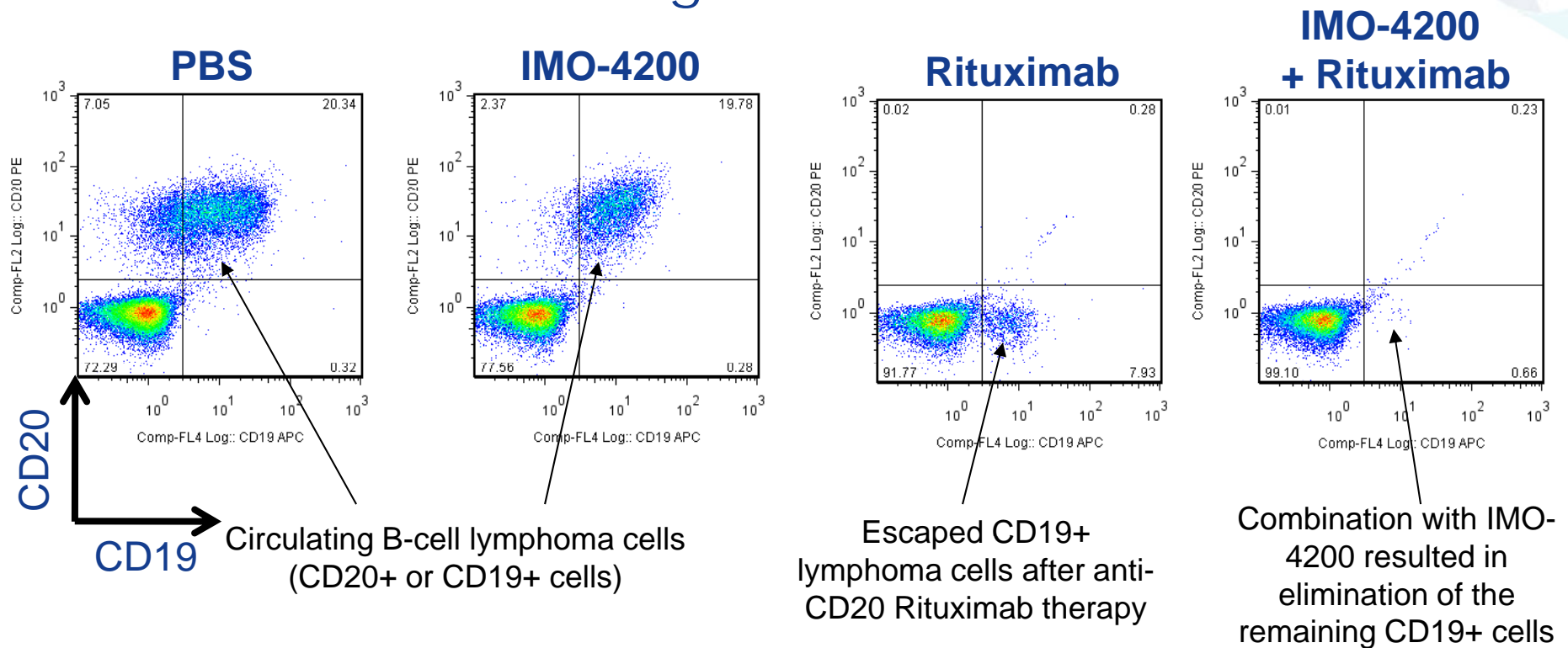
■ PBS ■ OFA
■ IMO-4200 ■ IMO-4200 + OFA



■ Untreated ■ RTX
■ IMO-4200 ■ IMO-4200 + RTX

- Raji: 1×10^6 cells, i.v., day 0
- Ofatumumab or Rituximab: 10 mg/kg, i.p., day 3, BIW for 5 times
- IMO-4200: 50 mg/kg, s.c., day 3, BIW for 10 times

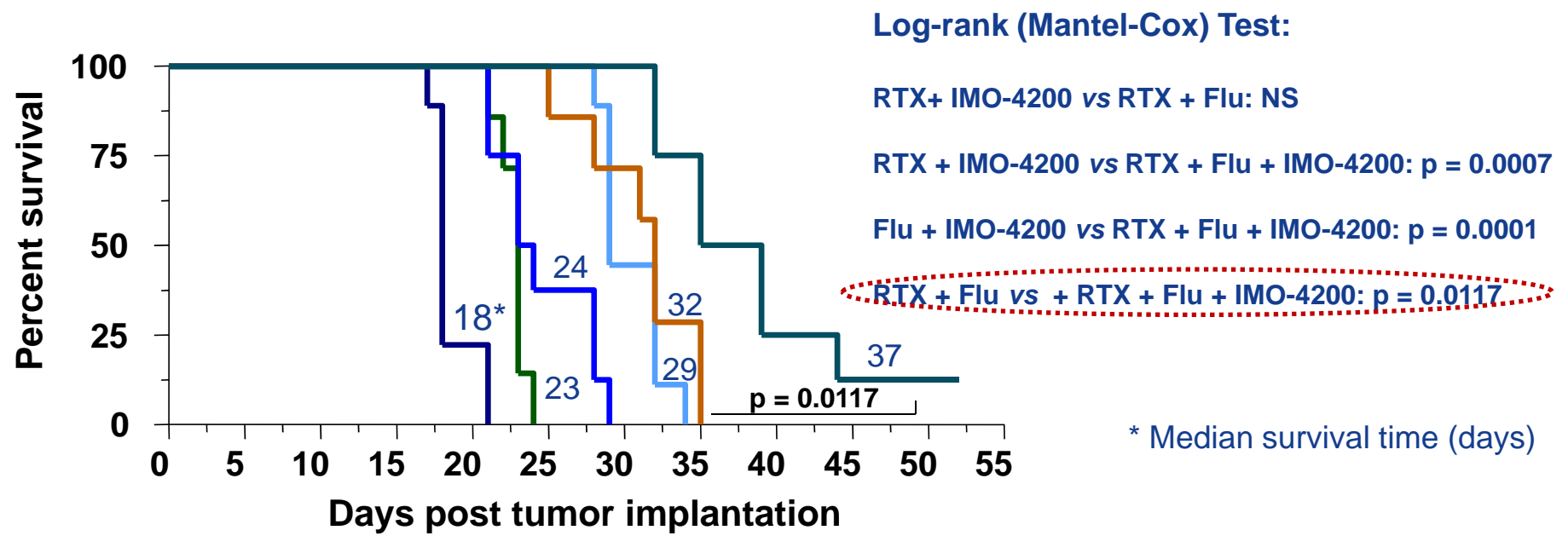
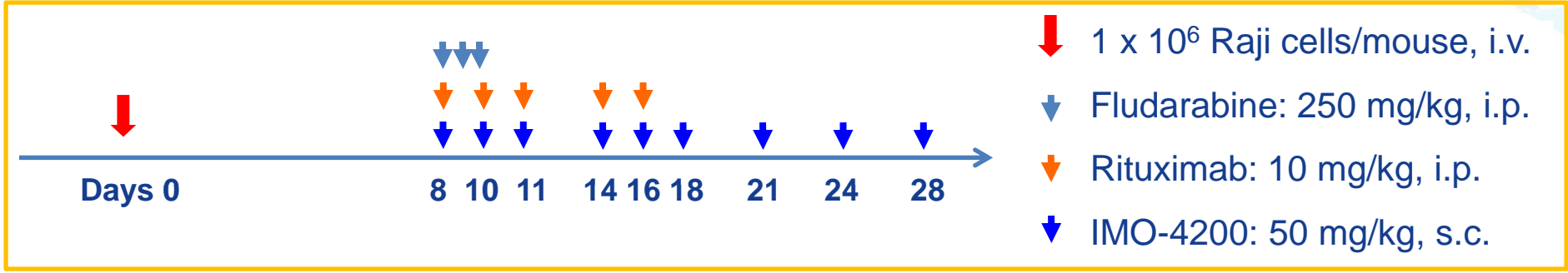
IMO-4200–Rituximab Combination Clears Circulating Tumor Cells



Circulating B-Cell Lymphoma Cells in the Peripheral Blood of the Mice Bearing HBL-2 Lymphoma

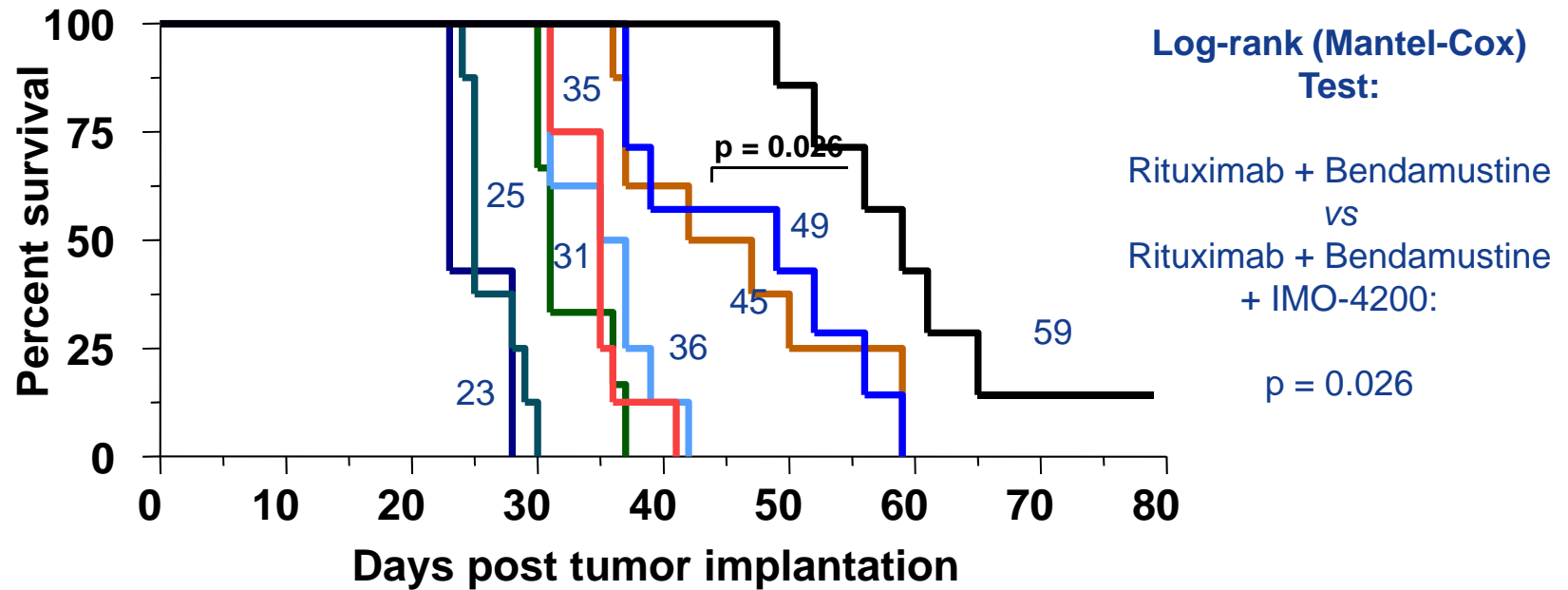
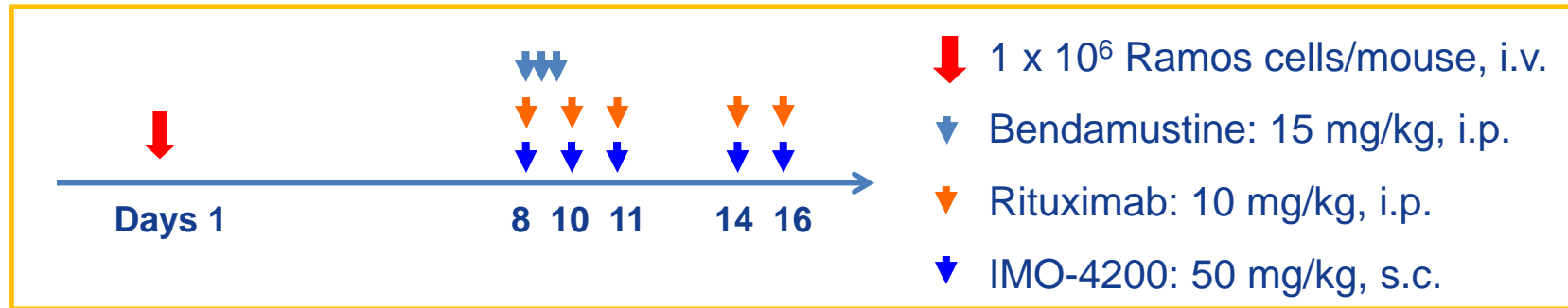
	% CD20+ Cells	% CD20-CD19+ Cells	% Total B Lymphoma Cells	% Inhibition
PBS	27.39	0.32	27.71	
IMO-4200	22.15	0.28	22.43	19.1
Rituximab	0.3	7.93	8.23	70.3
IMO-4200 + Rituximab	0.24	0.66	0.9	96.8

IMO-4200 Enhances Antitumor Effect of Rituximab and Fludarabine – Raji Xenograft Model



- PBS
- RTX
- RTX + IMO-4200
- FLU + IMO-4200
- RTX + FLU
- RTX + FLU + IMO-4200

IMO-4200 Enhances Antitumor Effect of Rituximab and Bendamustine – Ramos Xenograft Model



- PBS
 — IMO-4200
 — RTX
 — BMT
- RTX + IMO-4200
 — BMT + IMO-4200
- RTX + BMT
 — RTX + BMT + IMO-4200

Conclusions

- In preclinical studies the dual TLR7/8 agonist
 - Exerts significant antitumor effects in combination with rituximab and ofatumumab
 - Increases antitumor efficacy of rituximab combined with fludarabine or bendamustine
 - Enhances activation of NK cells contributing to increased rituximab-mediated ADCC
 - Increases efficacy of mAb-mediated CDC
- IMO-4200 identified as lead candidate for clinical development
 - Potential to combine with a range of therapeutic agents
 - For the treatment of hematological malignancies