Preclinical Studies of IL-21 Plus Rituximab Combination Therapy for B-Cell Lymphoma

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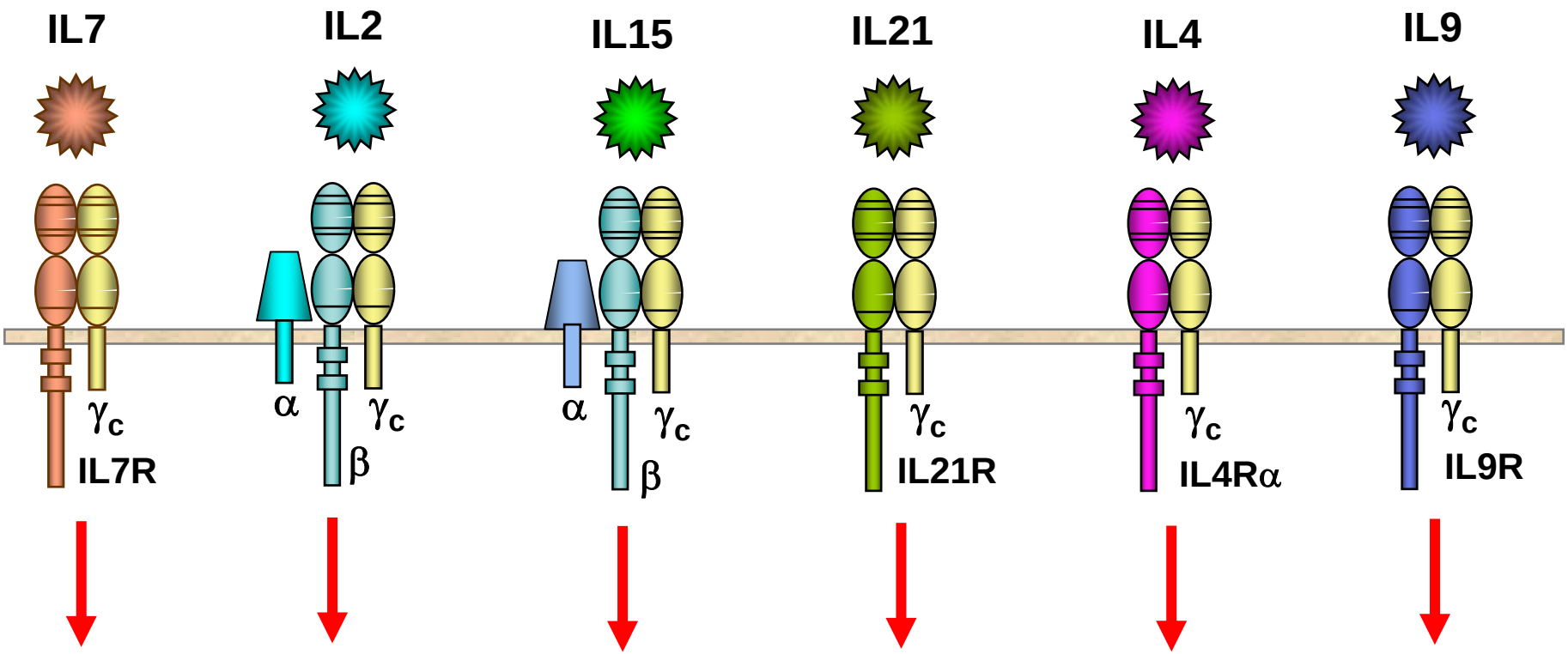
Cytokines that utilize the γ–Common Cytokine Receptor



Lymphocyte survival and Development	Th1; T- cell Homeostasis Cell-mediated Immunity	Th1; T-, NK cell Homeostasis Cell-mediated Immunity	?	Th2; Humoral Immunity B-cell regulation	Th2; Mast Cell Biology

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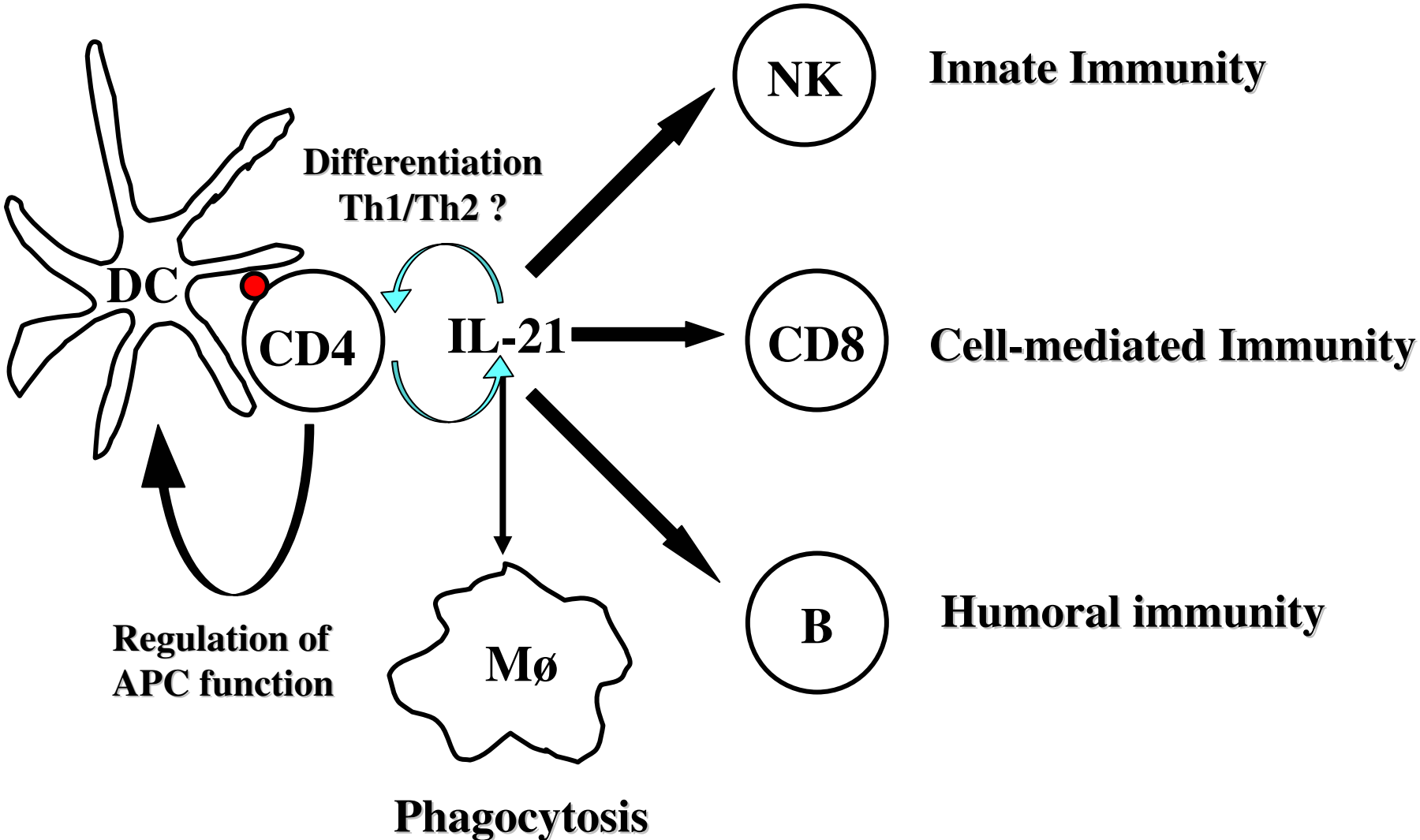
IL-21 is a CD4+ "T-Helper" cytokine that acts on most leukocyte subsets



Rationale for testing IL-21 in B-cell lymphoma

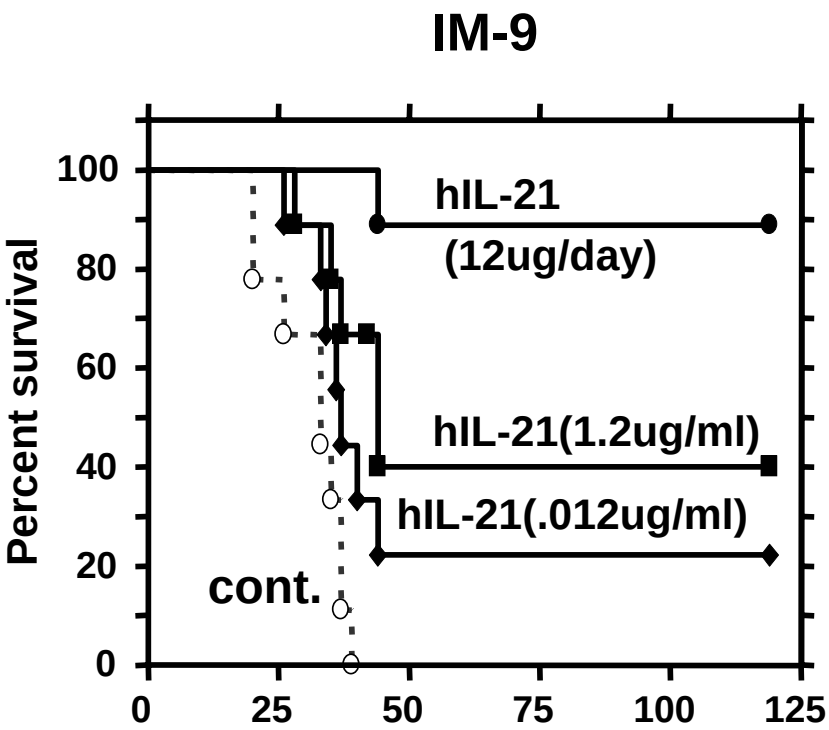
1. IL-21 is a potent regulator of B-cell differentiation (plasma cells) and can stimulate apoptosis of "bystander" B-cells.

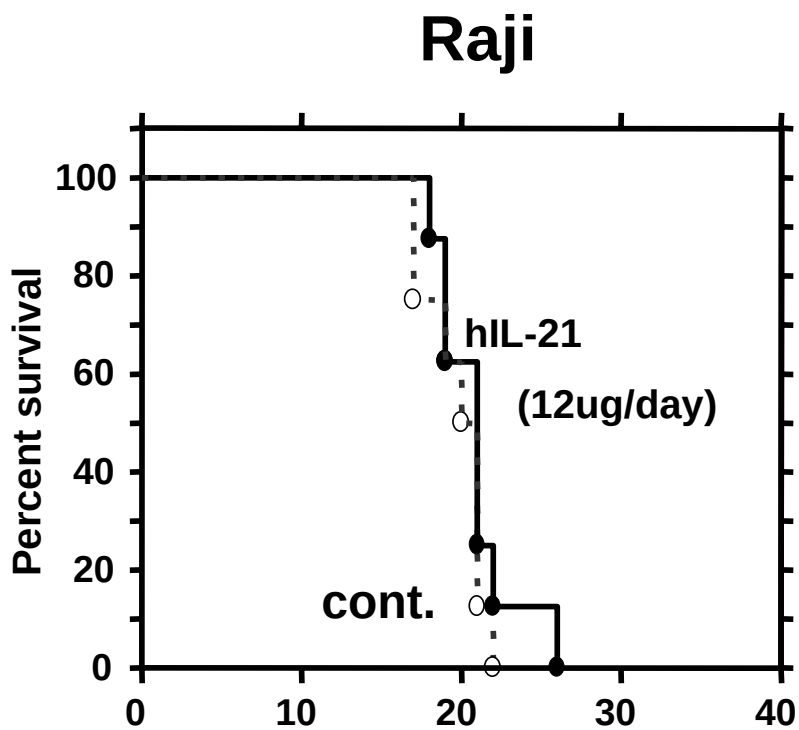
- **IL-21 inhibits the proliferation of many B cell tumor lines in vitro and in vivo.**

IL-21 inhibits the proliferation of B lymphoma cell lines in vitro.

Cell Type	Treatment duration (d)	Percentage Change (Mean ± sd)	Number of Experi-ments
WSU-NHL	12	-82.3 ± 6.1	4
IM-9	8	-77.1 ± 18.1	5
MC116	11	-62.7 ± 6.2	5
HS-Sultan	9	-49 ± 38.2	4
Raji	8	-13 ± 2.7	2
Ramos	12	-7.4 ± 7.1	3
DOHH2	10	-10.1 ± 17.8	7

IL-21 prolongs survival of mice with disseminated human B lymphoma





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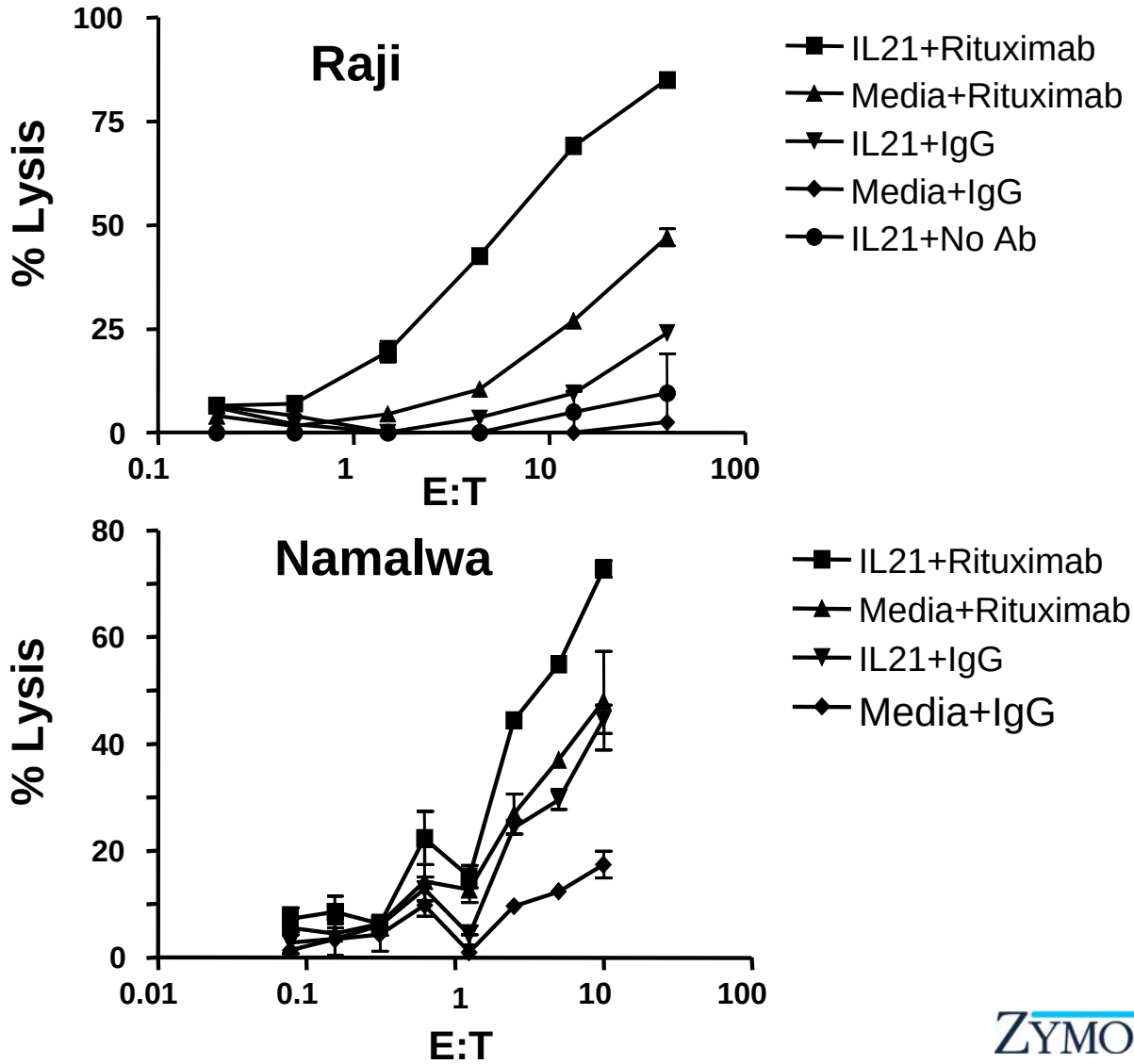
Rationale for testing IL-21 in the context of B-cell lymphoma

1. Direct actions on B cells and NHL

2. IL-21 enhances Rituximab-mediated anti-tumor activity.

IL-21 enhances antibody dependent tumor cell killing by NK cells in vitro



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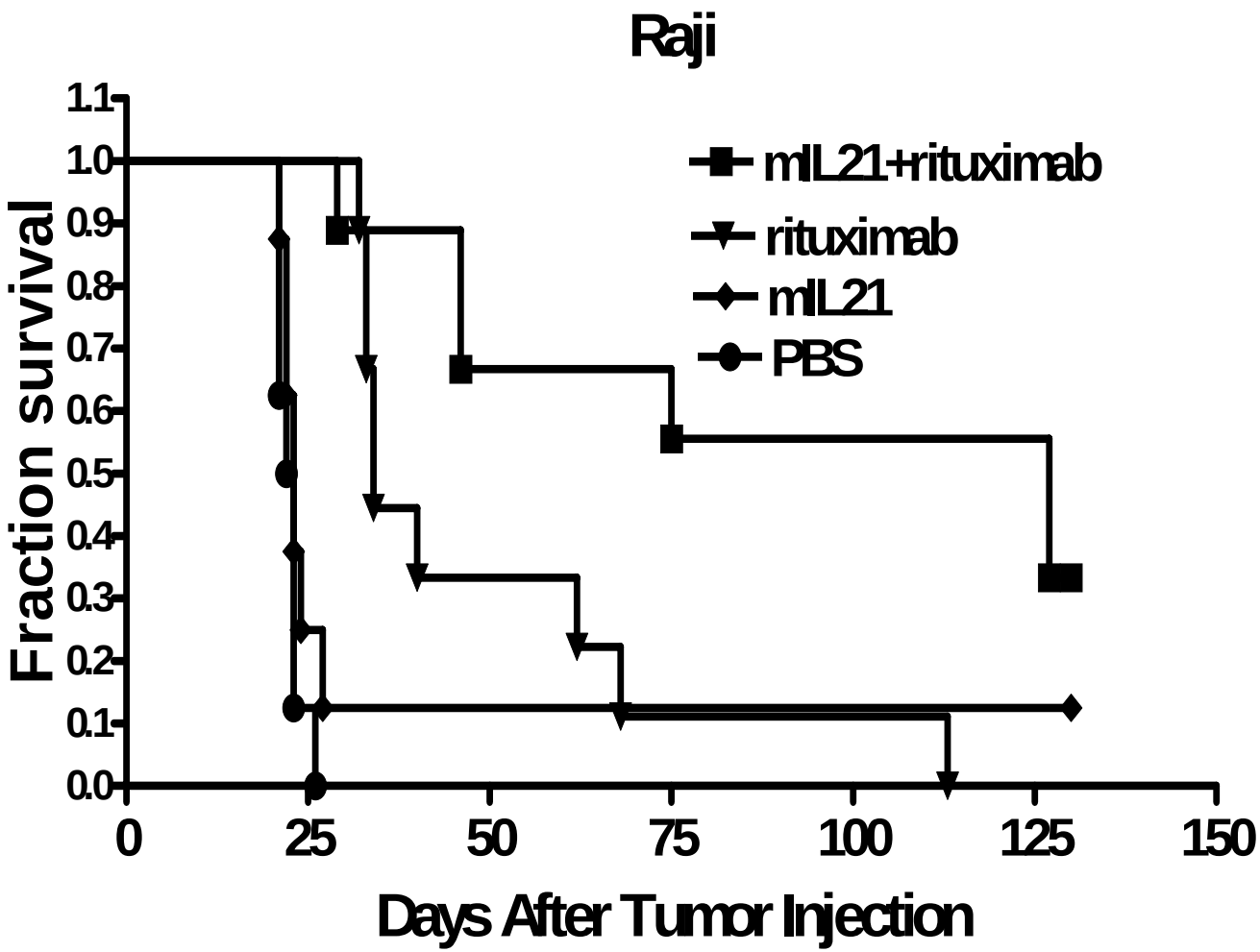
IL-21 enhances rituximab-mediated survival of lymphoma-bearing mice



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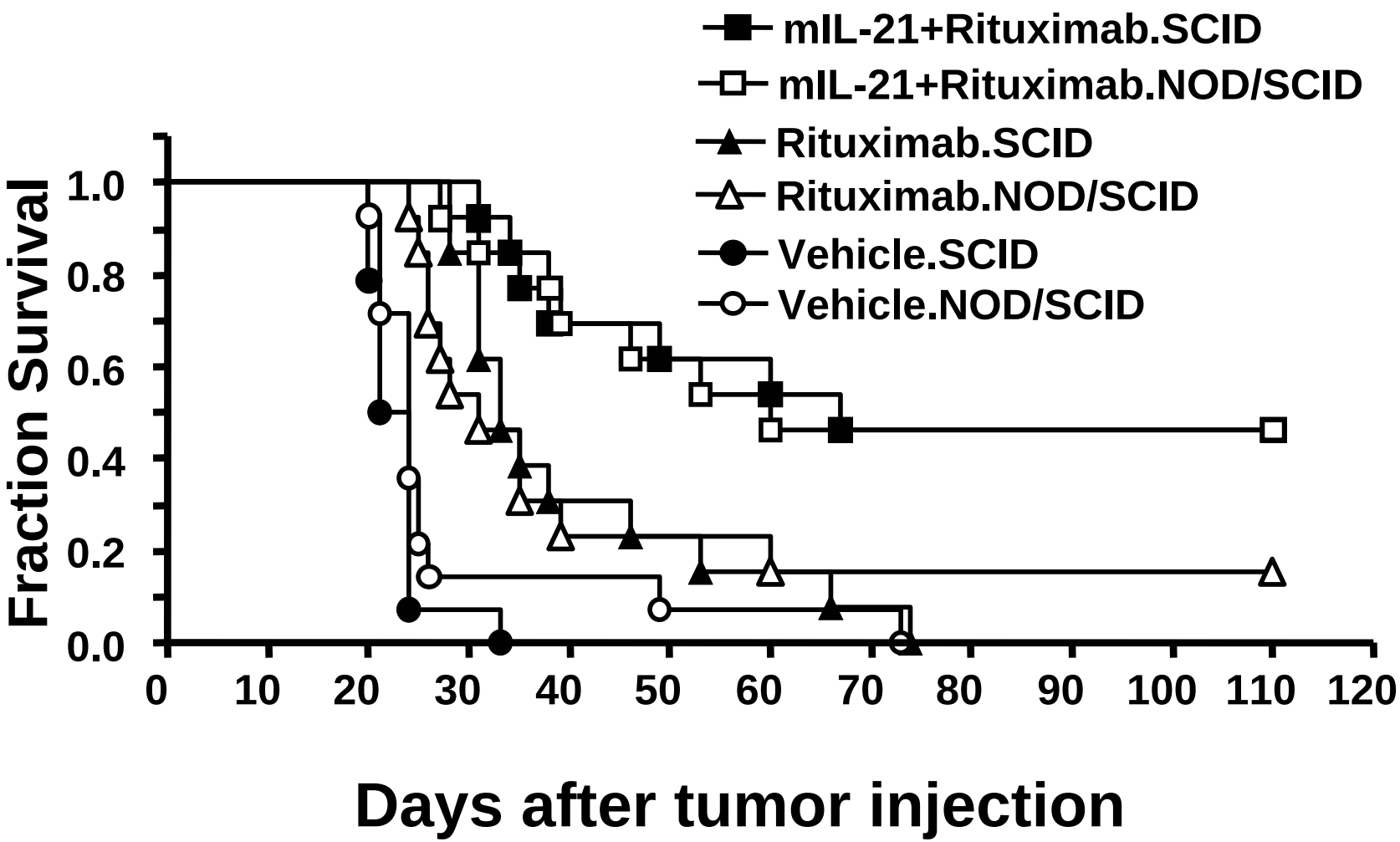
IL-21 enhances rituximab activity in the absence of functional NK cells



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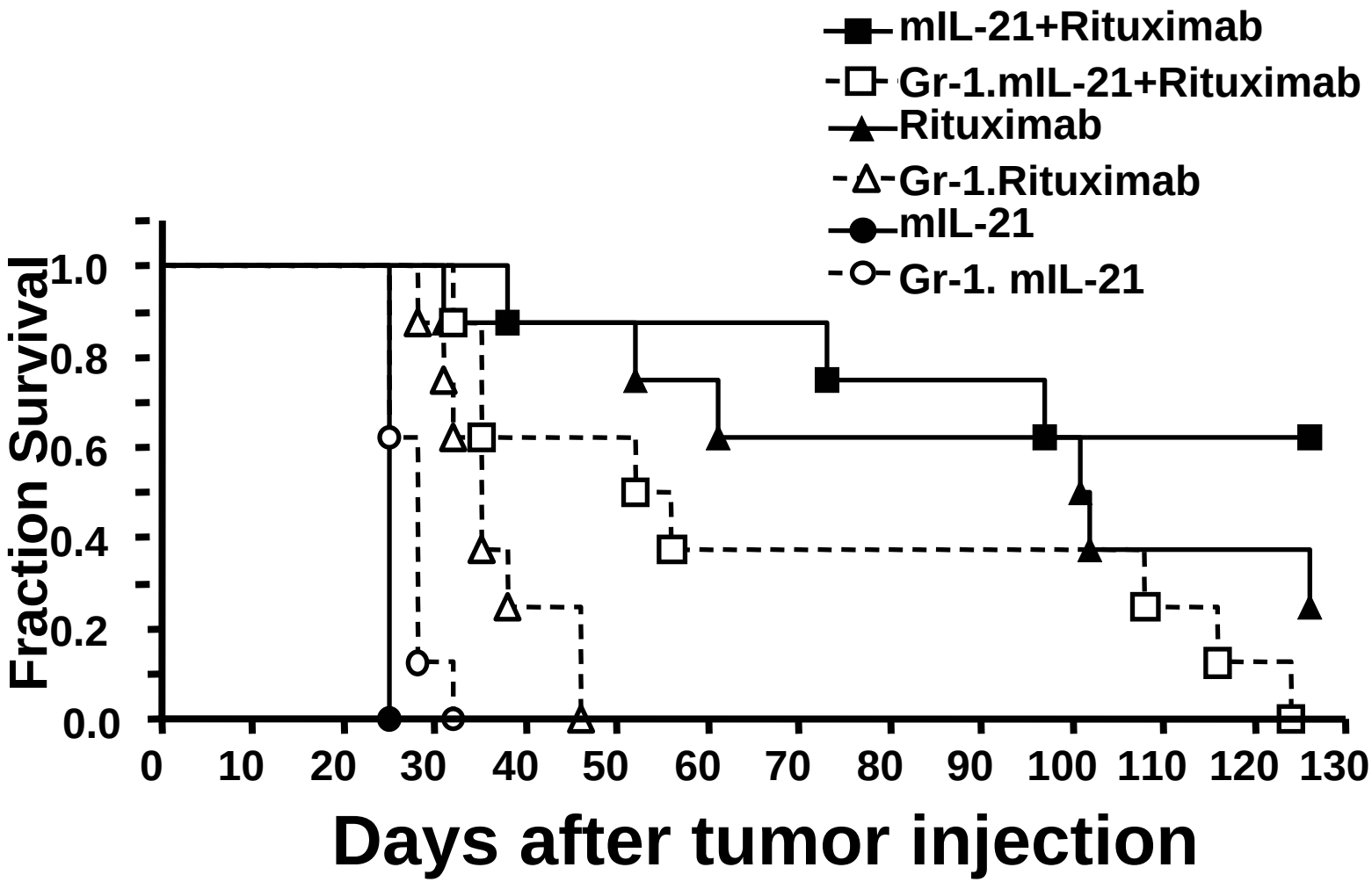
Survival of tumor-bearing mice treated with IL-21+ rituximab is partially regulated by Granulocytes



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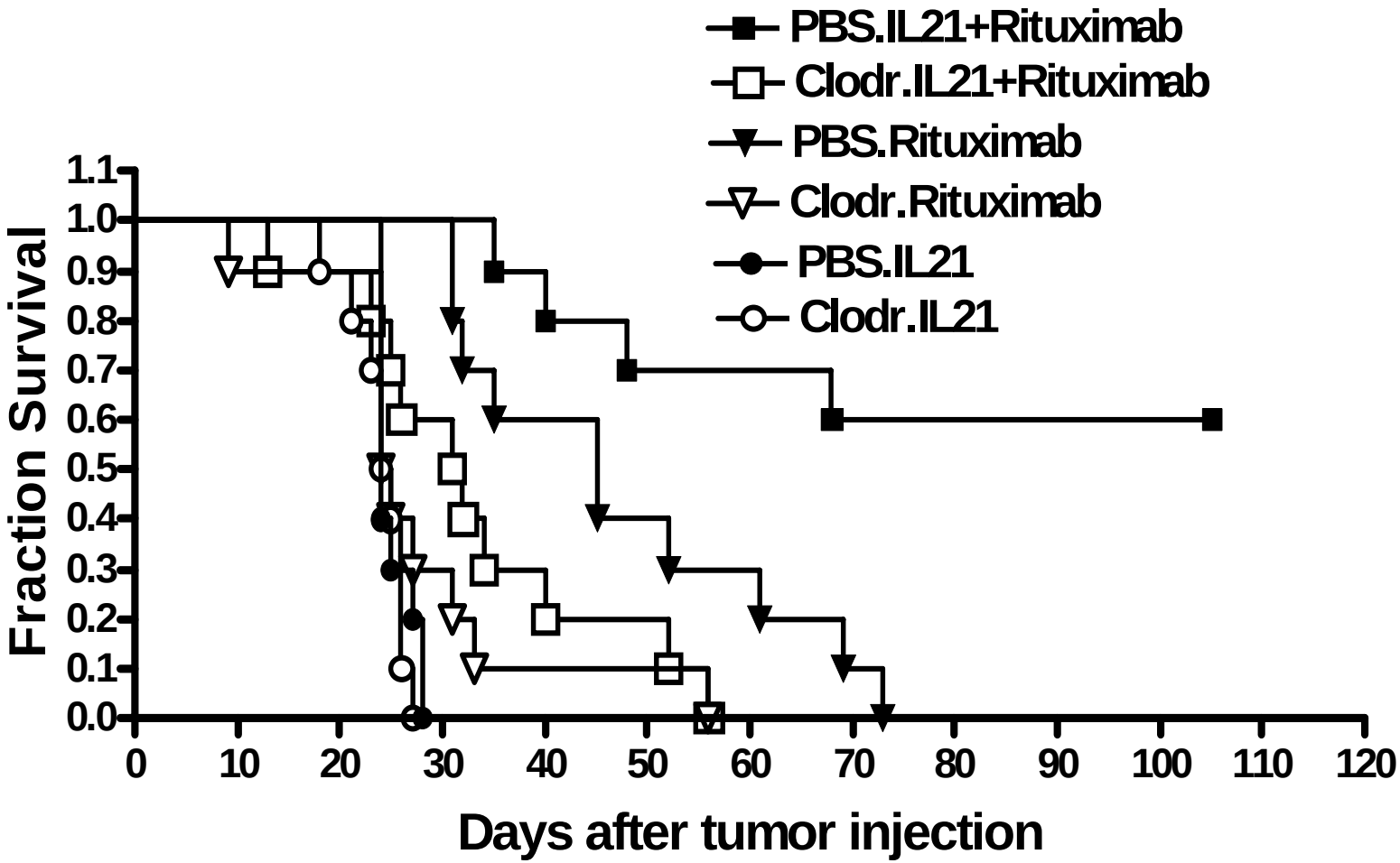
Survival of tumor-bearing mice treated with IL-21 +Rituximab is partially regulated by Macrophage



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Rationale for testing IL-21 in the context of B-cell lymphoma

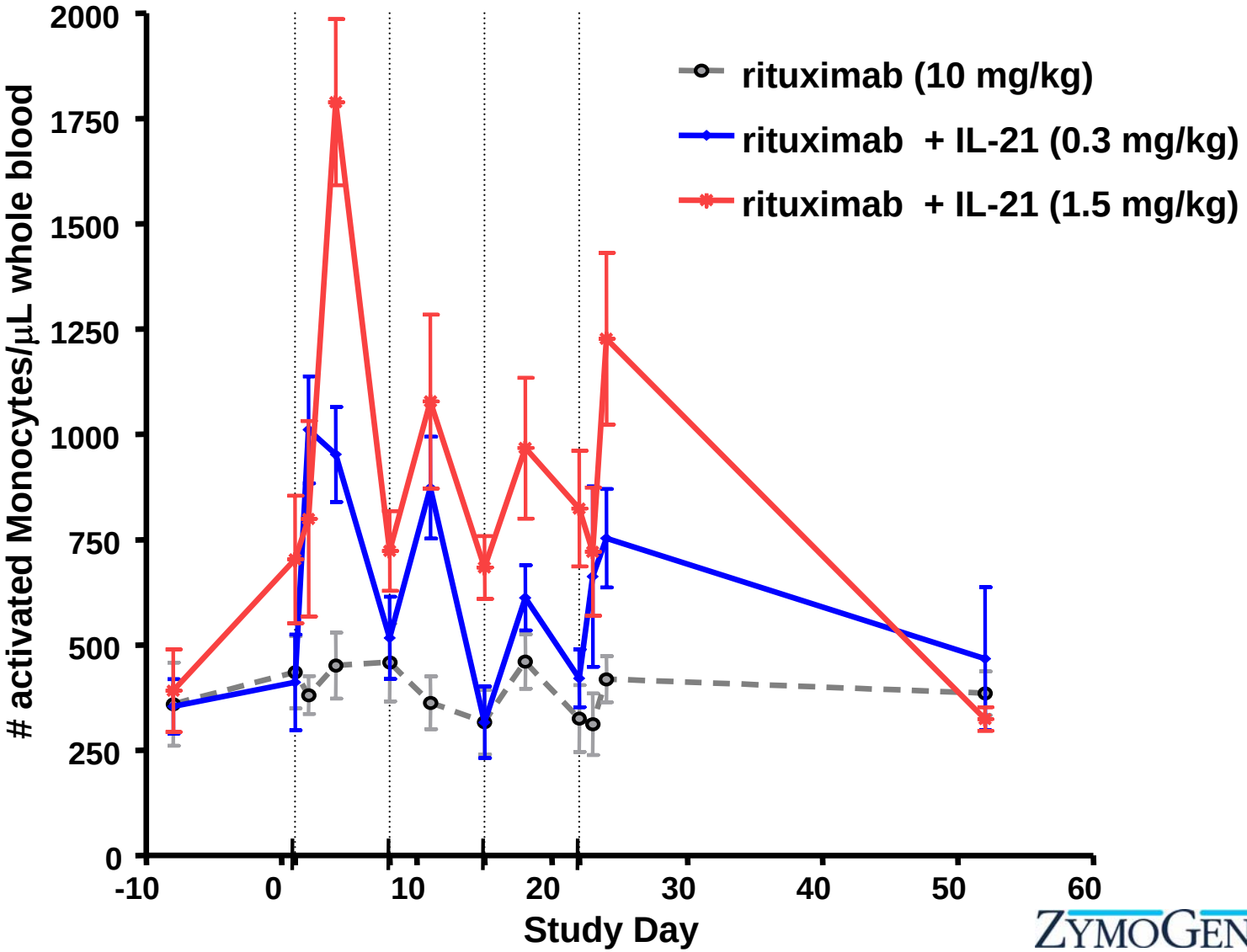
1. Direct actions on B cells and NHL
2. Stimulation of anti-tumor immunity
3. IL-21 enhances rituximab-mediated anti-tumor activity in mice.
4. **IL-21 prolongs rituximab-mediated B-cell depletion in non-human primates.**

Study Groups

Group	Rituxan	rIL-21	Animals Terminal Sac	Animals Recovery Sac
1	10 mg/kg Once weekly	0 mg/kg	4	4
2	10 mg/kg Once weekly	0.3 mg/kg Once weekly	4	4
3	10 mg/kg Once weekly	1.5 mg/kg Once weekly	4	4

Peripheral Activated Monocytes

$CD45^+/CD14^+/CD64^+$



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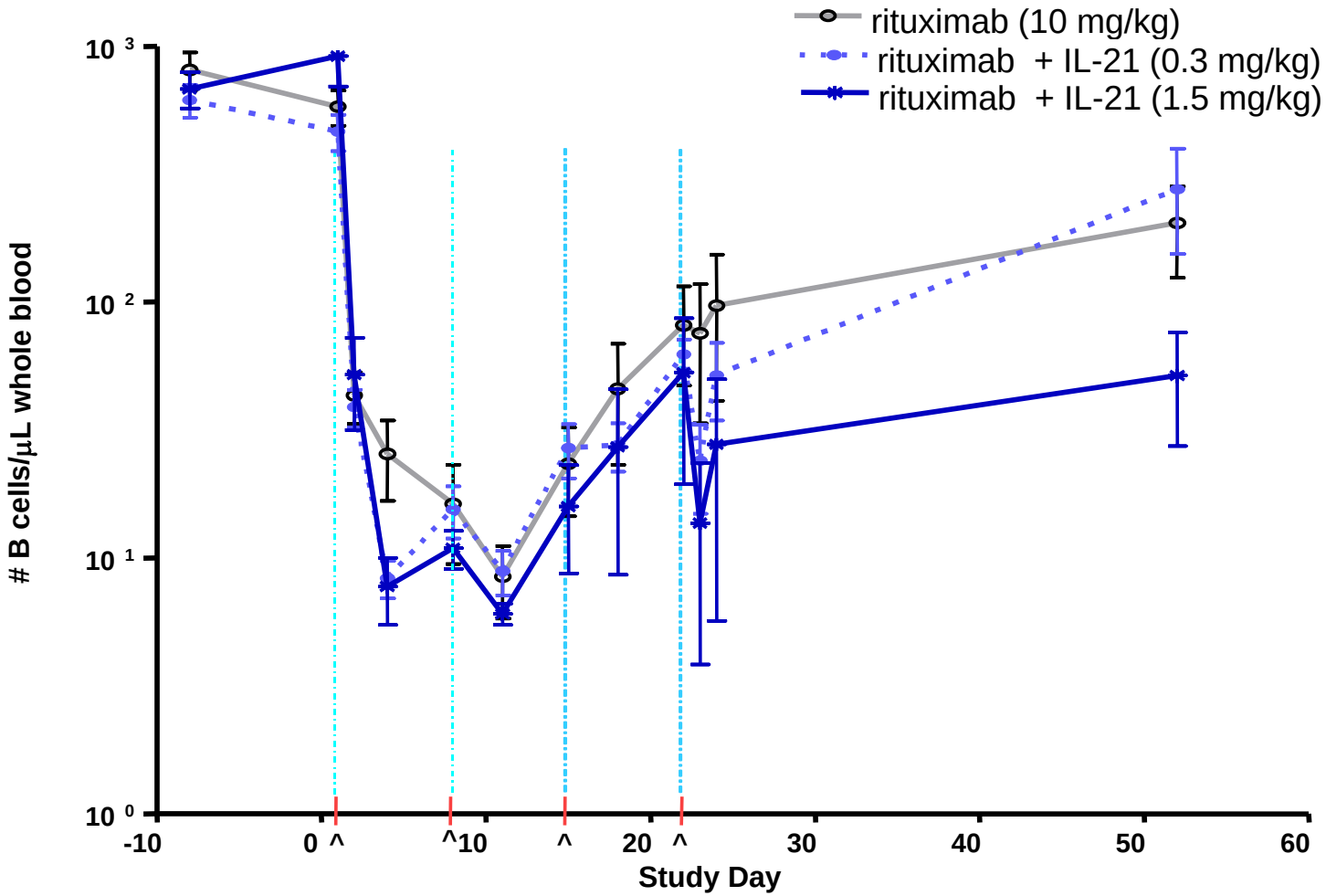
Group Mean B cells in Blood



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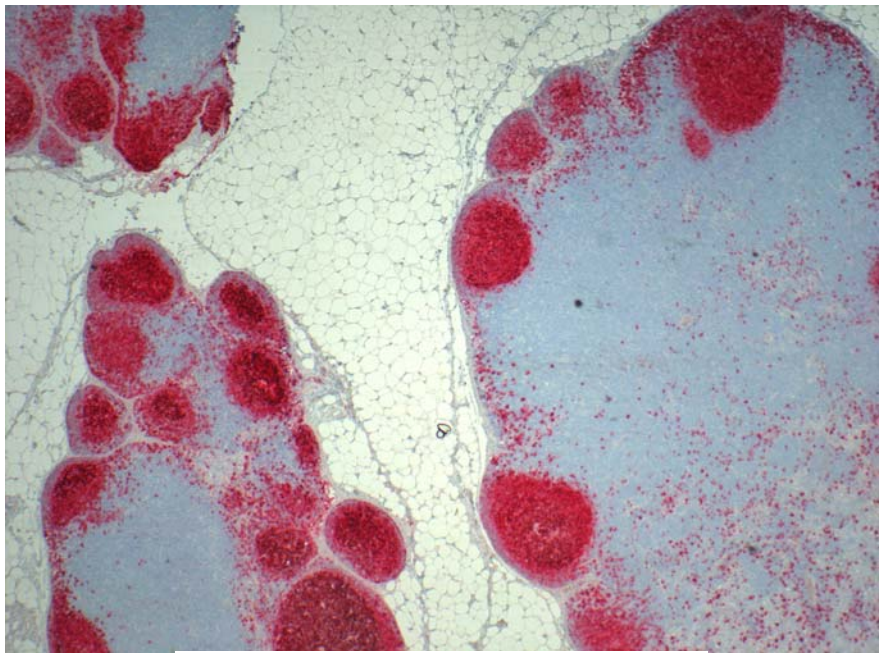
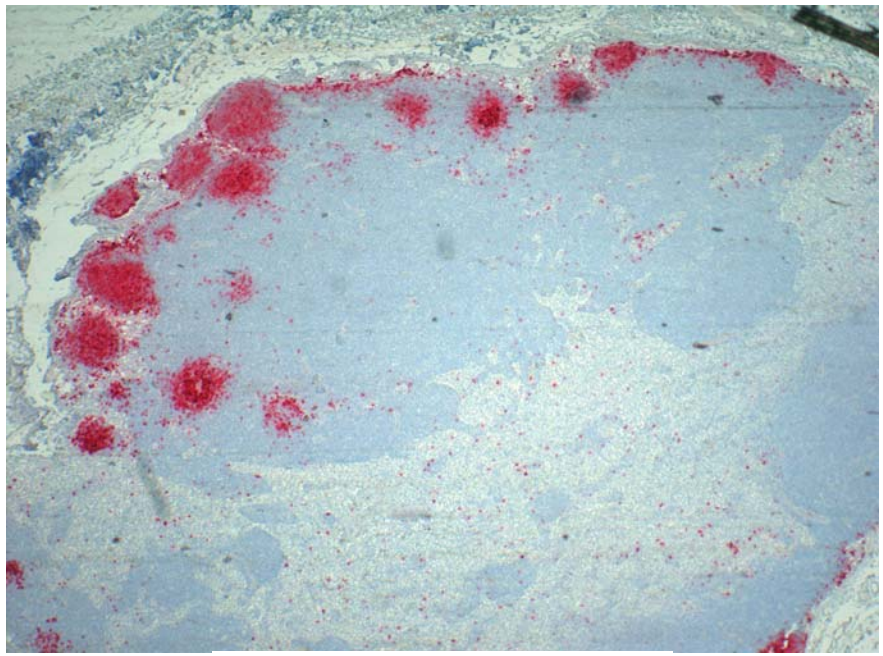
Lymph Node: CD20 IHC

CD20-stained B cells in Mesenteric Lymph Nodes



Rituximab only group
(4-week Recovery)



IL-21 + Rituximab
(4-week Recovery)

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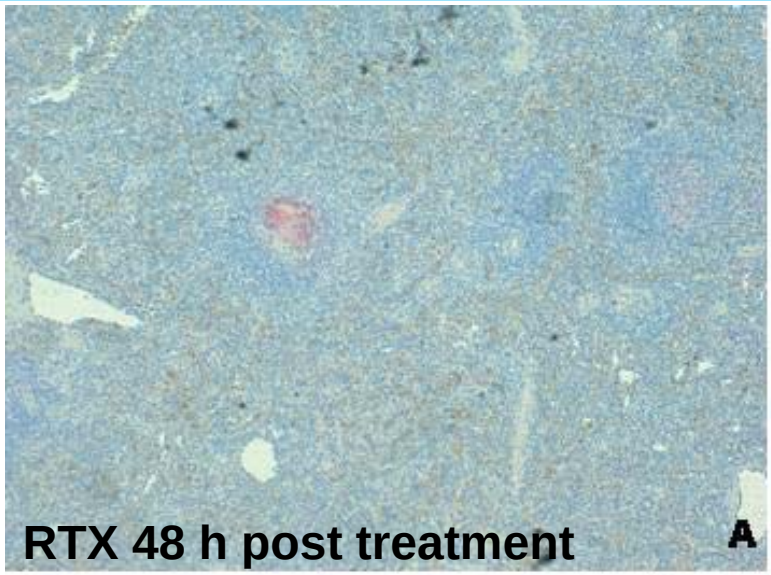
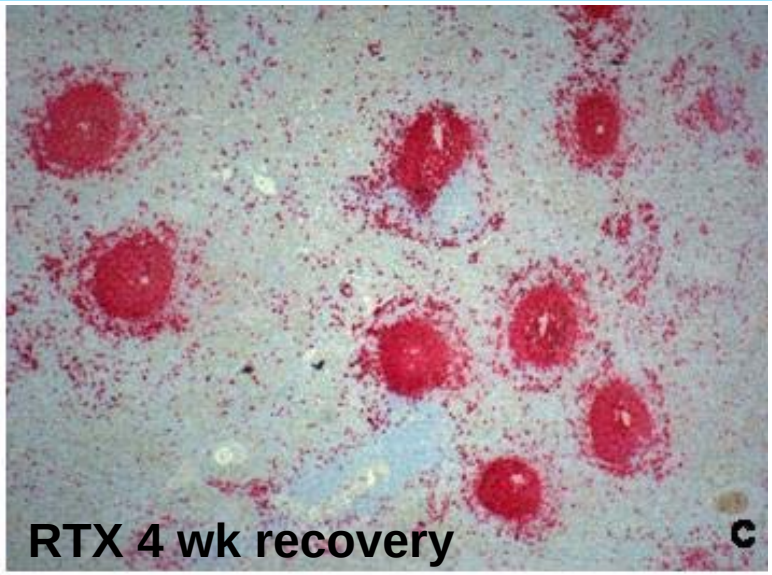
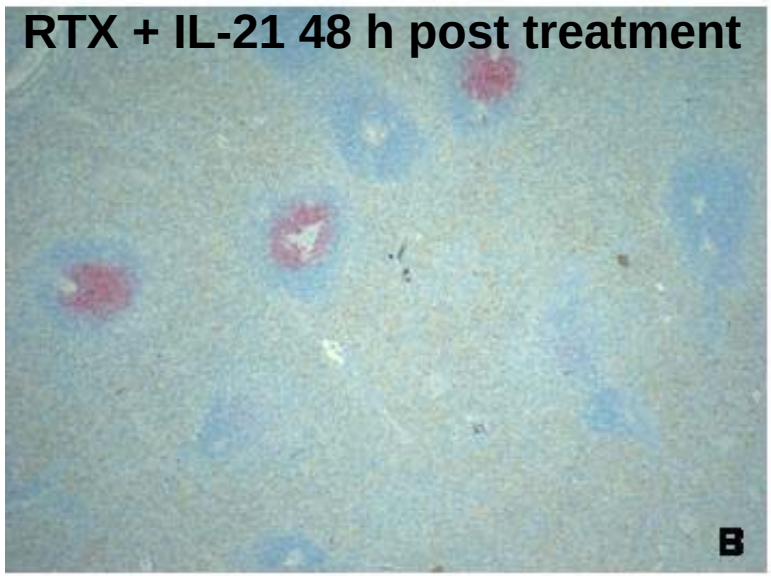
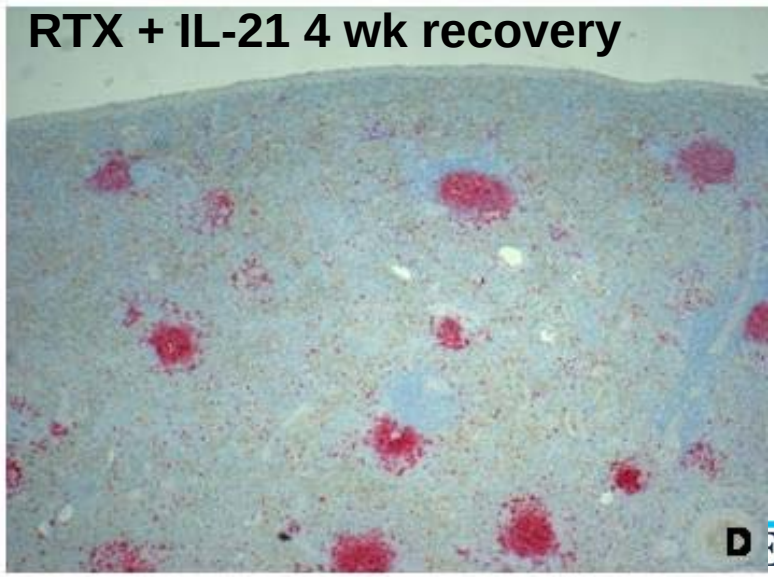
Spleen: CD20 IHC



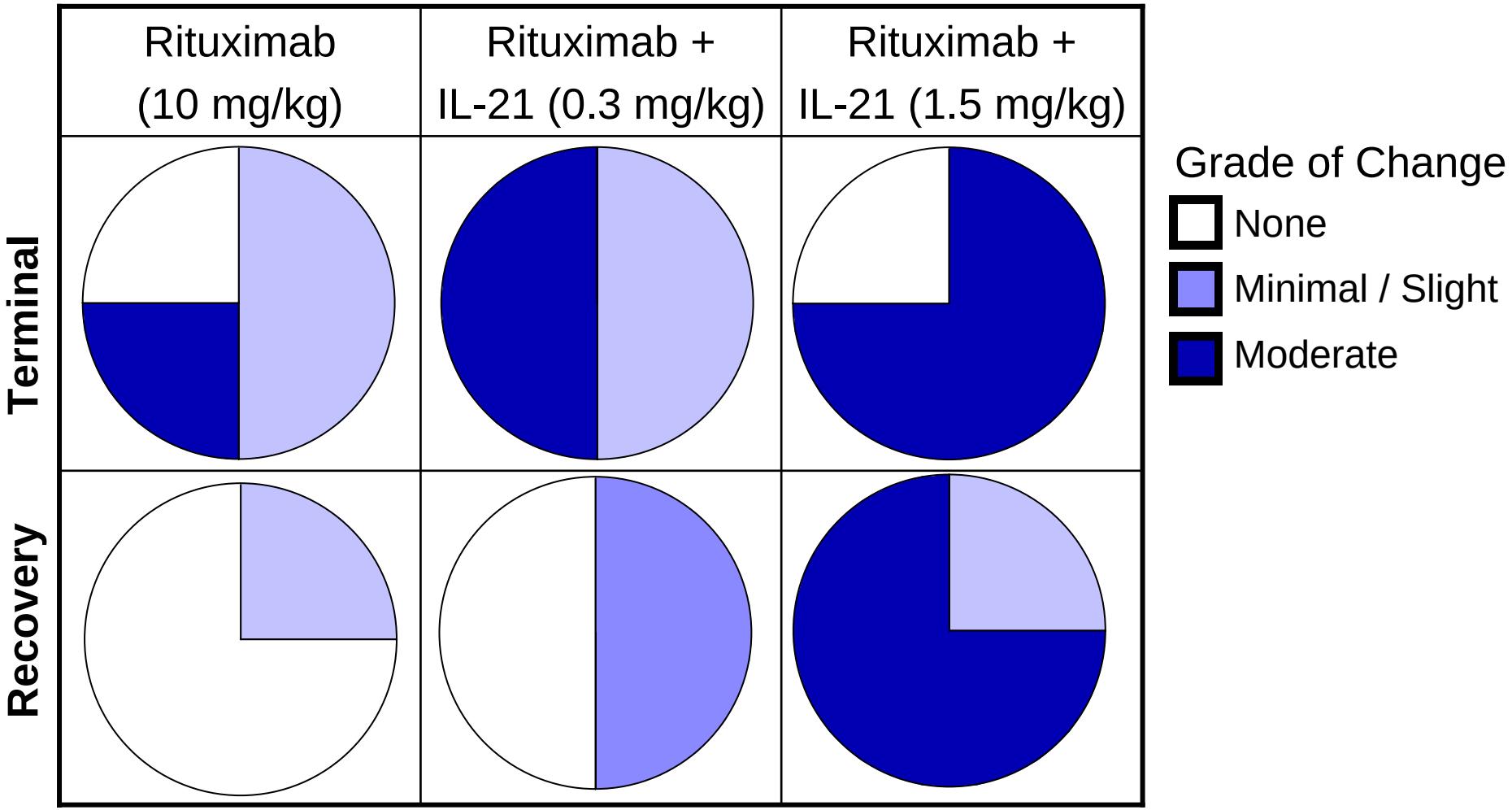






ETICS

Lymphoid Atrophy in Spleen



Summary

- IL-21 has direct antiproliferative activity on B lymphoma cell lines in vitro and in vivo.
- IL-21 enhances rituximab mediated killing of B lymphoma cell lines in vitro and in vivo.
- Prolongation of survival by rituximab + IL-21 is dependent on granulocytes and macrophage.
- IL-21 causes monocyte activation and prolonged B cell depletion in nonhuman primates.

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Rafael Ponce
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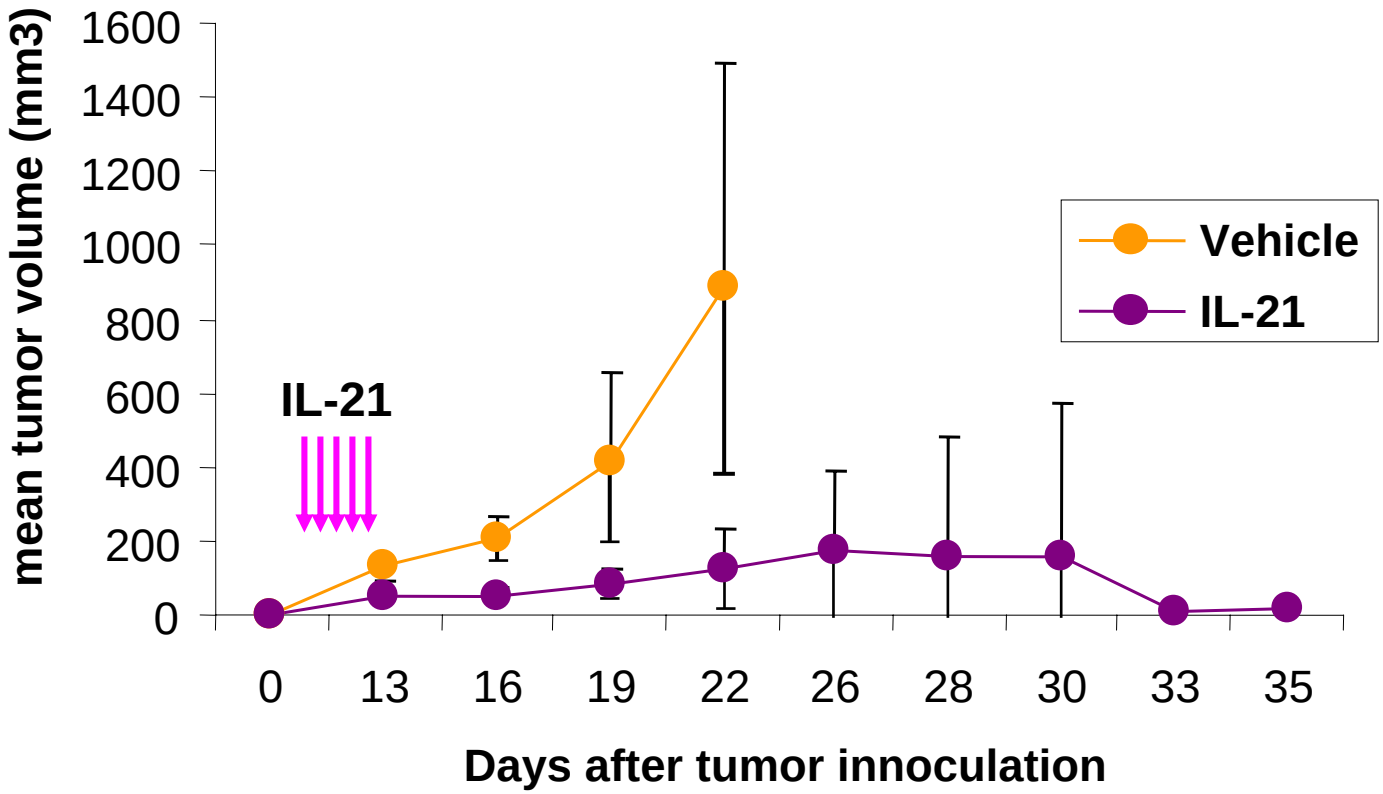
IL-21 inhibits growth of RenCa.2 tumors



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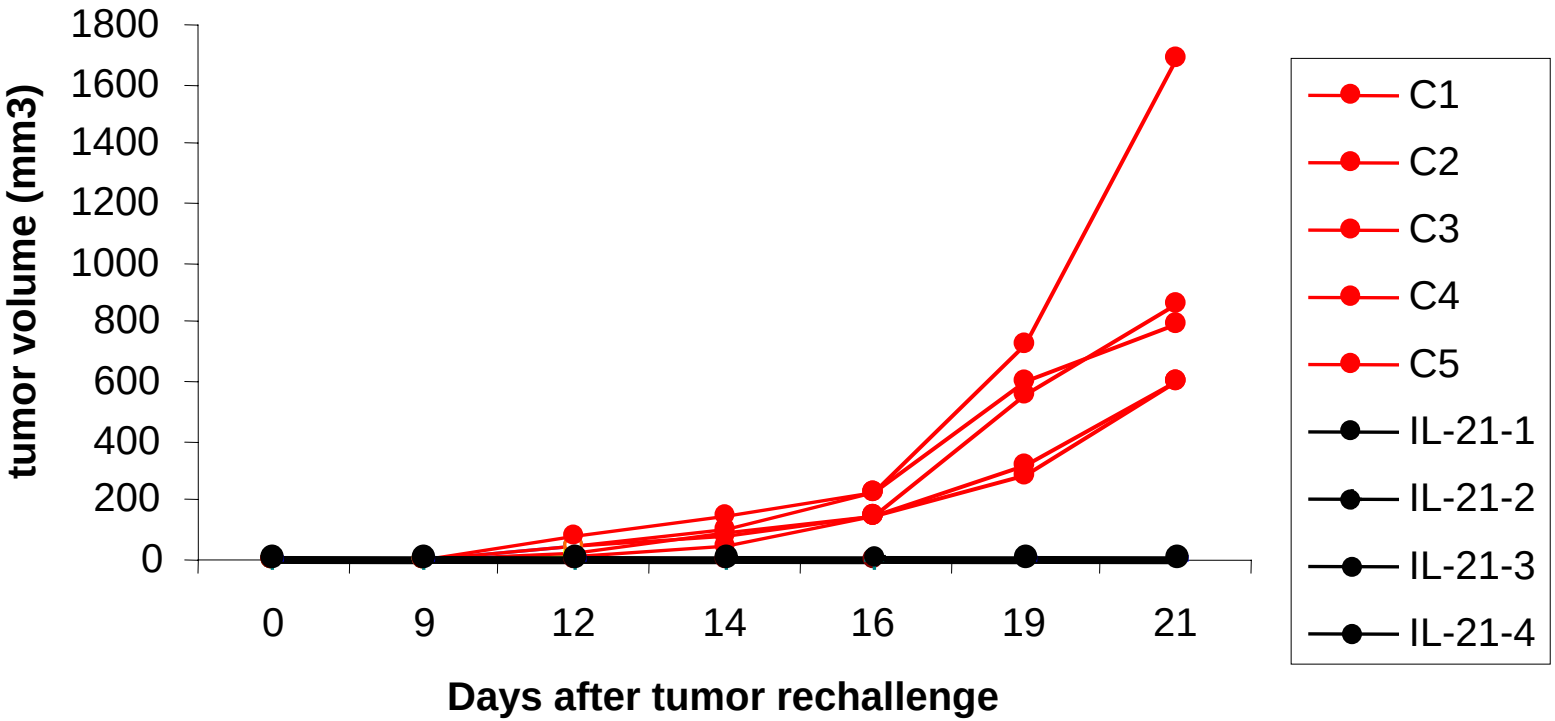
Establishment of memory in Renca.2tumor-bearing mice



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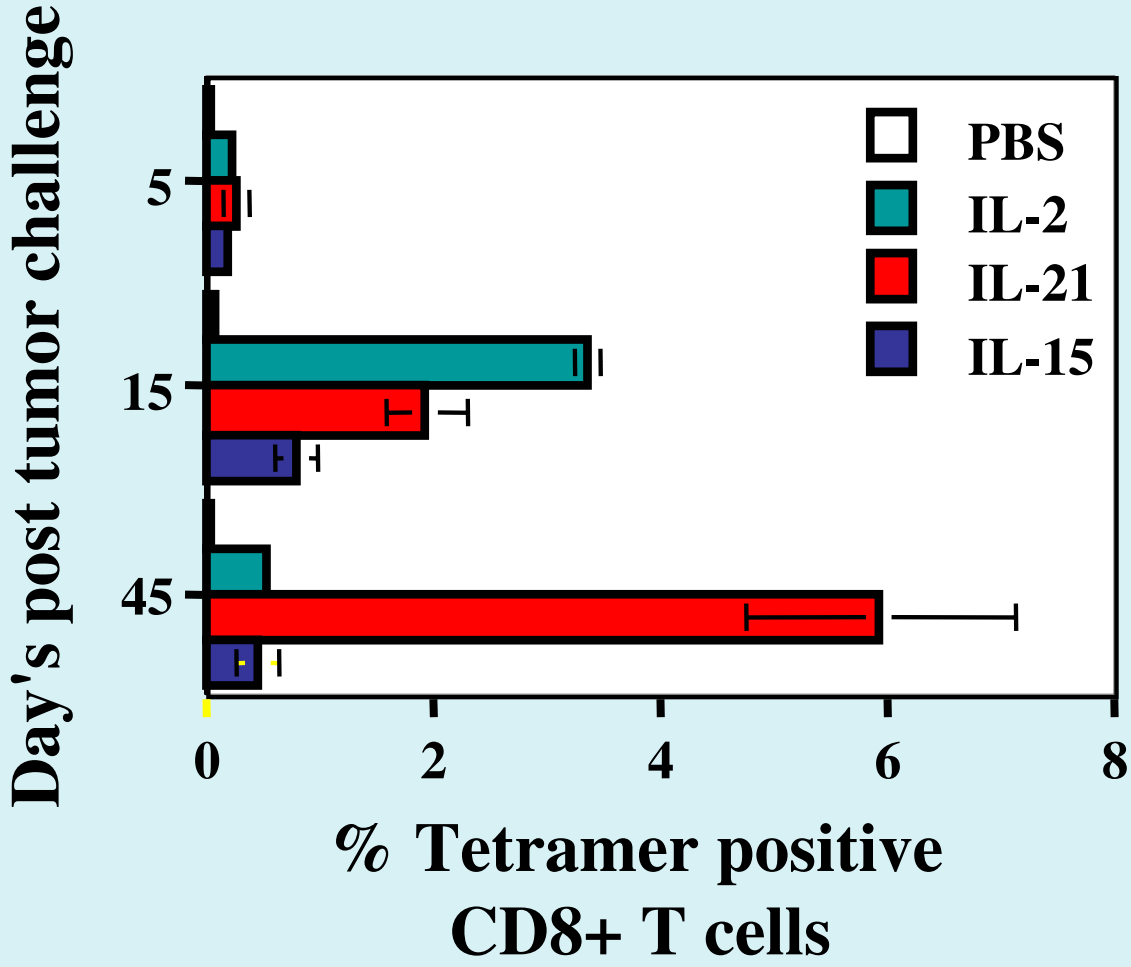
IL-21 Induces and Sustains CD8+ T-cells Responses to E.G7



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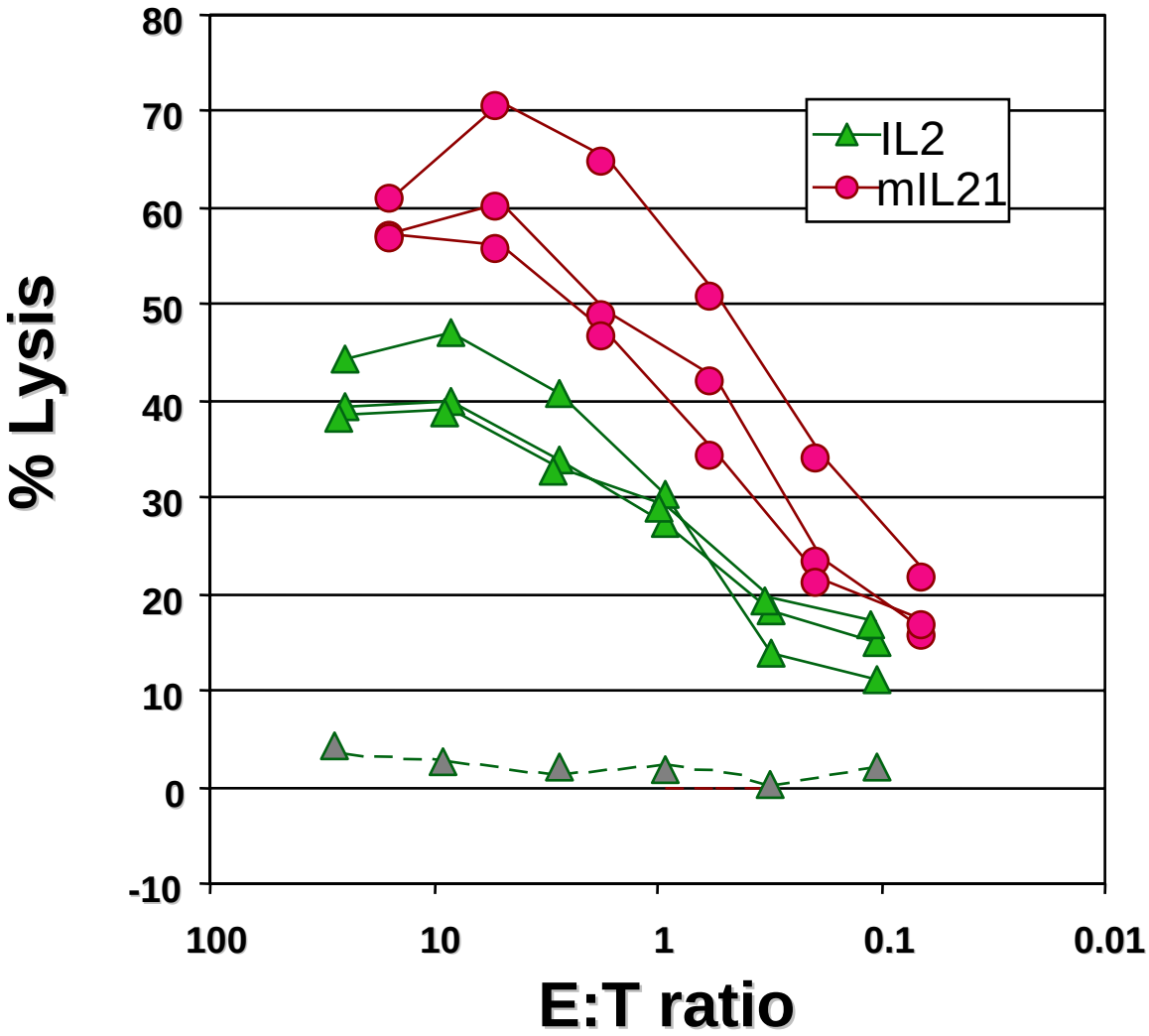
IL-21 Enhances Tumor Killing Cytotoxic T Cells



Target = EG.7 mouse thymoma

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Antitumor activity of IL-21

Tumour	IL-21 treatment	Effect on tumour	Mechanism
B16 melanoma or MCA205 fibrosarcoma	Injection of IL-21-encoding plasmid at day 5 and day 12 after tumour initiation	Increases survival rate (40%)	NK-cell-mediated killing, partially $CD8^+$ T-cell mediated
B16 melanoma or MethA fibrosarcoma	Expression of IL-21 by tumour	Prevents initiation of tumour (for 41 weeks), protects from later tumour challenge (results in delayed growth)	Tumour-specific CTL and NK-cell activity, perforin dependent
Mouse colon carcinoma or human pancreatic carcinoma	Expression of IL-21 by tumour	Prevents initiation of tumour	NK-cell-mediated killing
TS/A mammary adenocarcinoma	Expression of IL-21 by tumour	Prevents initiation of tumour, protects from later tumour challenge (results in delayed growth)	$CD8^+$ T-cell- and granulocyte-dependent mechanism, partially IFN-γ dependent
RLmale1 lymphoma	Injection of IL-21-encoding plasmid before or after lymphoma injection	No effect on the number of metastatic foci, synergistic protective effect when in combination with IL-15	$CD4^+$ T-cell-, $CD8^+$ T-cell- and NK-cell-dependent mechanism
B16 melanoma	Injection of IL-21-encoding plasmid before tumour injection	Reduces lung and liver metastases	NK-cell-mediated killing, perforin but not IFN-γ dependent
E.G7 thymoma (ovalbumin expressing)	Injection of IL-21 (20 µg intraperitoneally) early, during days 2–12 after tumour initiation	Increases survival compared with administration of IL-2 or IL-15	$CD8^+$ T-cell-mediated mechanism
	Injection of IL-21 (20 µg intraperitoneally) late, during days 12–22 after tumour initiation	Protects from later tumour challenge	Increased persistence of tumour-specific $CD8^+$ T cells
B16 melanoma	Injection of IL-21 (5–10 µg intraperitoneally twice daily), and adoptive transfer of tumour-specific $CD8^+$ T cells at day 8–10 after tumour initiation	Cures established tumours, synergistic effect when in combination with IL-15	$CD8^+$ T-cell-mediated killing, IL-21 and IL-15 mediate functional and proliferative changes in $CD8^+$ T cells

CTL, cytotoxic T lymphocyte; IFN-γ, interferon-γ; IL, interleukin; NK, natural killer.

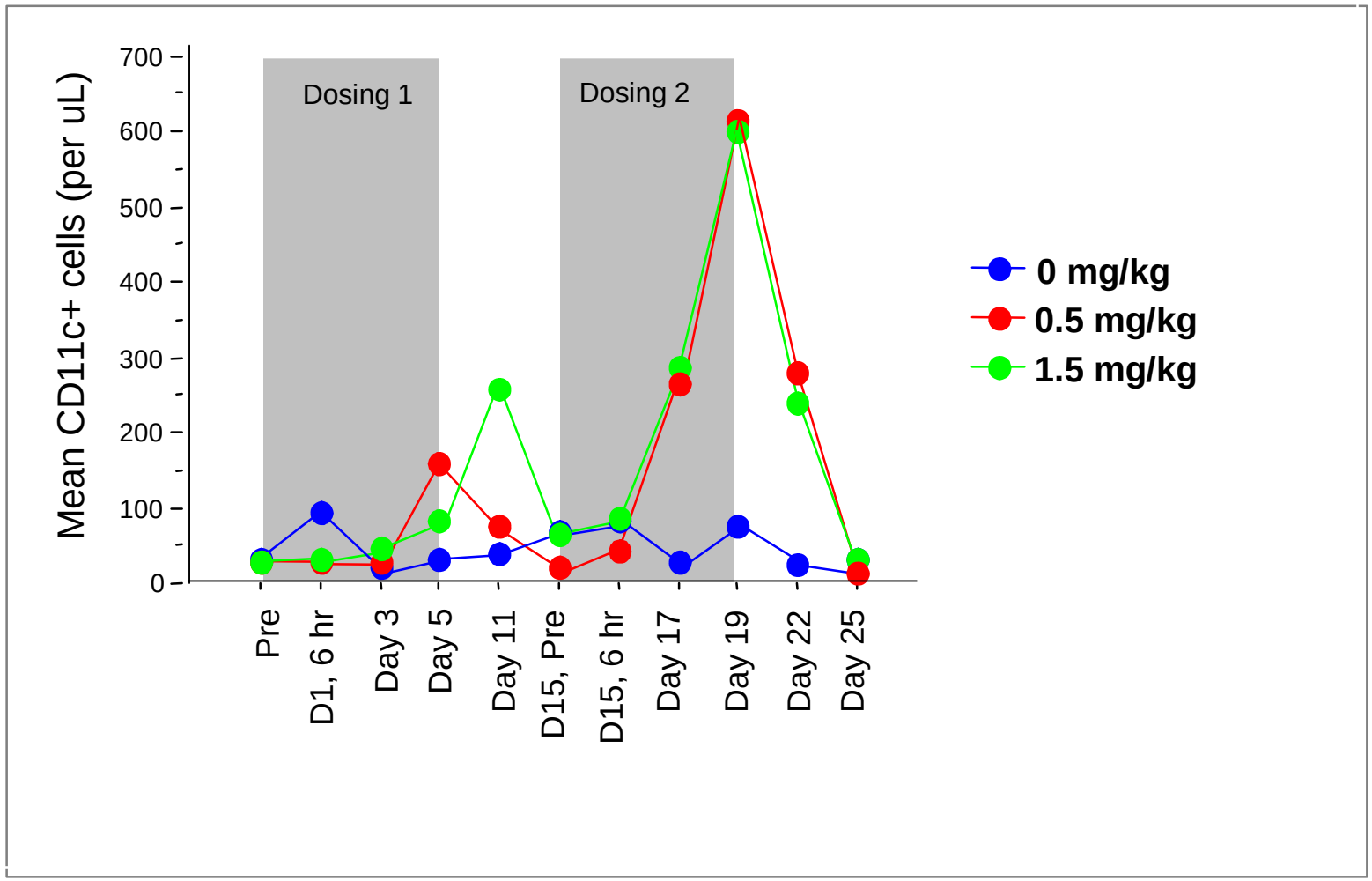
Leonard and Spolski (2005)
Nat. Rev. Immunol.

Rationale for Testing IL-21 in the Context of B-cell Lymphoma

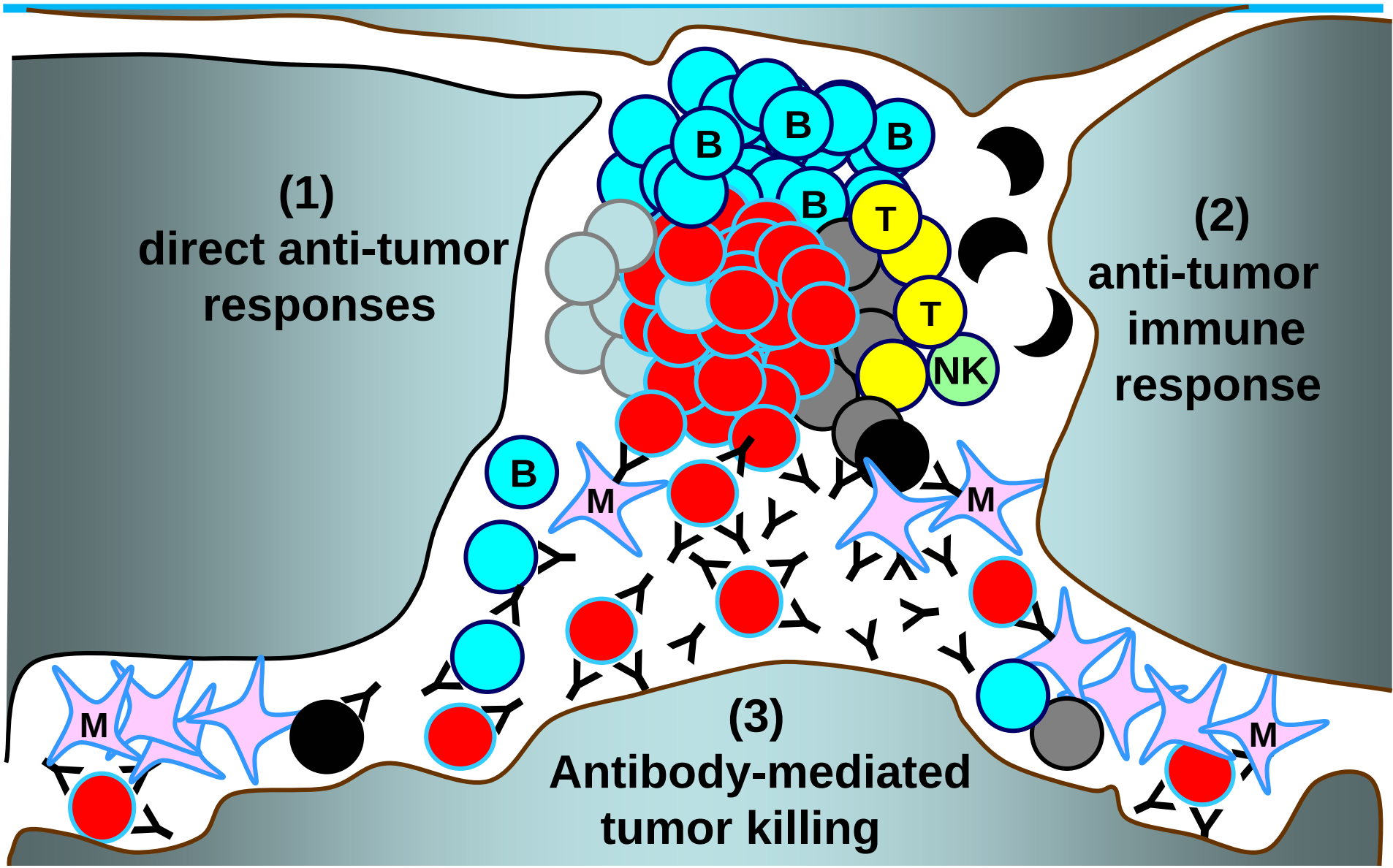
1. Direct actions on B cells and NHL

2. IL-21 is a potent regulator of anti-tumor immunity

IL-21 Elevates Myeloid/Monocyte Cells in Blood of Cynomologous Monkeys (CD11c+, per mL)



Summary


(1)
direct anti-tumor
responses
B
B
B
B
T
T
NK
(2)
anti-tumor
immune
response
B
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M
M
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(3)
Antibody-mediated
tumor killing