STING[$] agonists: storm-inciting stings?

Florian Colbatzky, Oct. 2018

[$] **St**imulator of **in**terferon **g**enes

Boehringer Ingelheim

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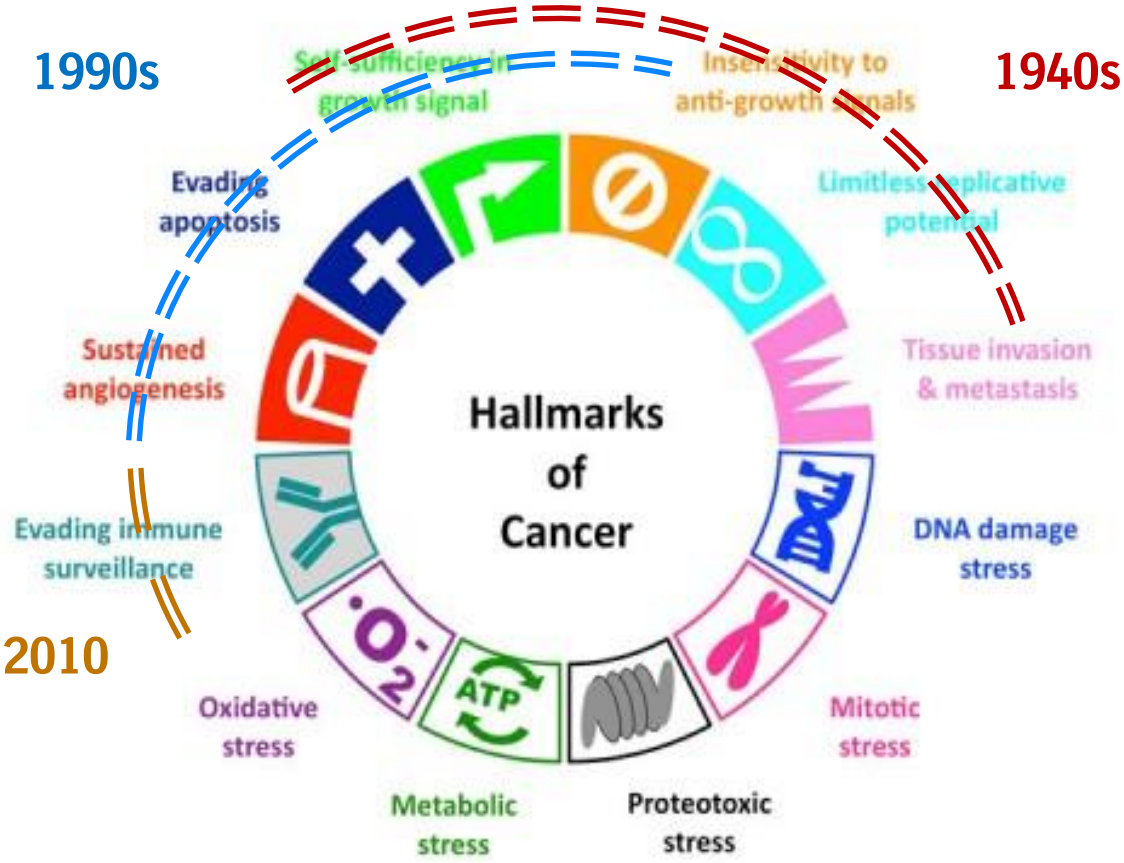
Concepts of anti-cancer treatment

Targeted therapy
- Rituximab
- Herceptin
- Gleevec

1990s

1940s

Chemotherapy
- Nitrogen Mustard
- Folic acid



Hallmarks of Cancer: modified from Hannahan & Weinberg, Cell 2011

Immunotherapy
- Yervoy
- Provenge

2010

Systemic therapies for treatment of patients with advanced and metastatic cancers

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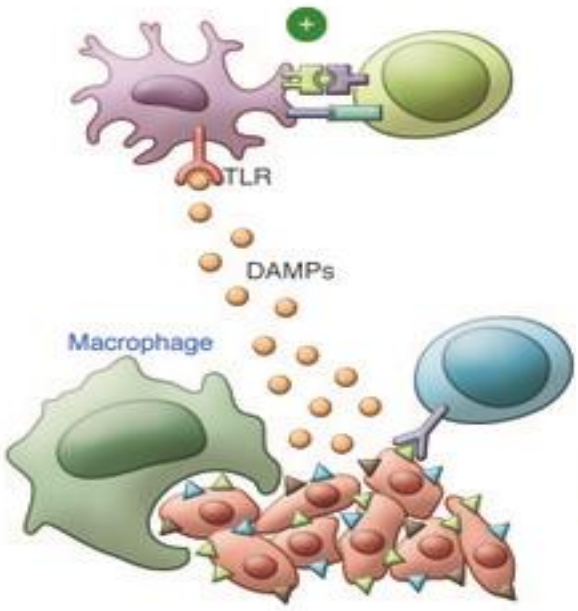
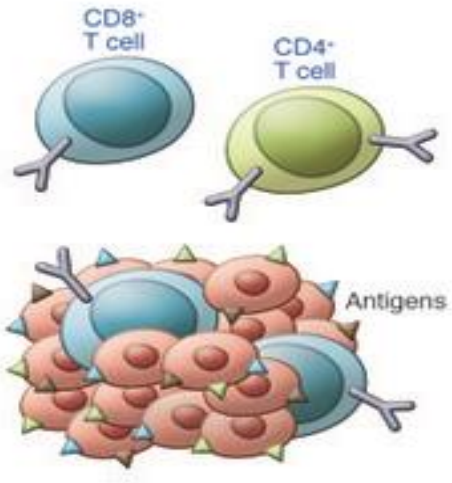
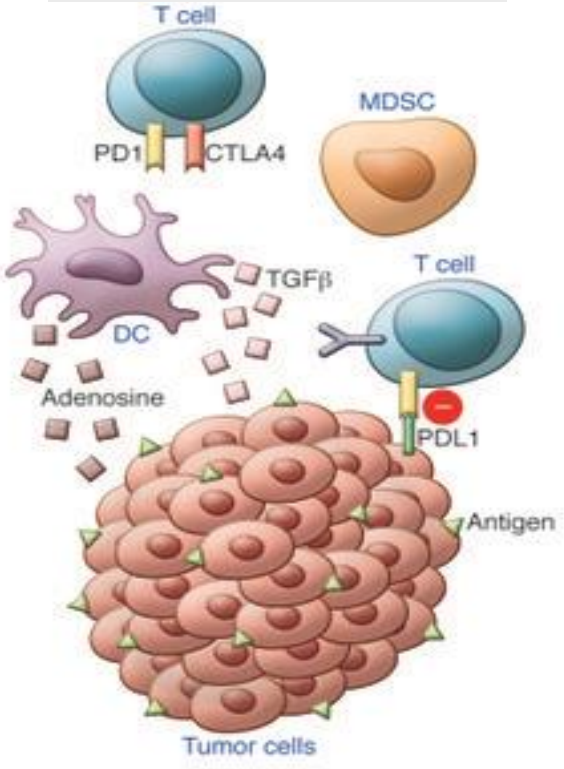
Concepts of anti-cancer treatment – immunotherapy

Elimination	Equilibrium	Escape
		
Innate and adaptive immune cells eliminate cancer cells	Dormant tumors with a dominant lymphoid compartment	Immune editing helps tumor cells to escape immune surveillance

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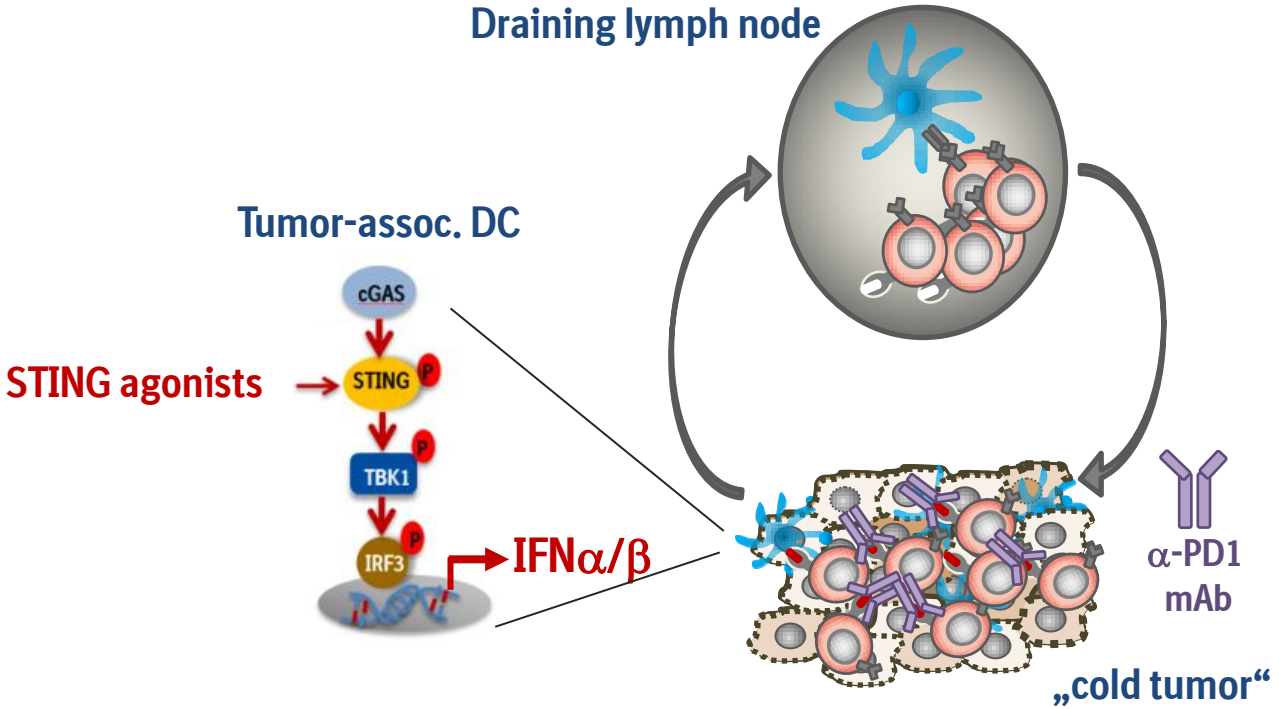
Therapeutic Concept



Local effect on dendritic cells

- Activation of dendritic cells
- Cytokine release
- Attraction of Killer T-cells

Systemic immune system activation

- Activation of dendritic cells
- Tumor antigen presentation
- T-cell activation against tumor protein

- MoA: The cytosolic DNA-STING pathway is a major mechanism for innate immune sensing of cancer and infection.

  STING agonists induce type I IFN secretion in tumor-associated DCs (and other innate immune cells) leading to an improved priming and activation of tumor-specific cytotoxic $CD8^+$ T cells and long-term immune surveillance.

- Treatment of patients with “non-inflamed or low-inflamed tumors”

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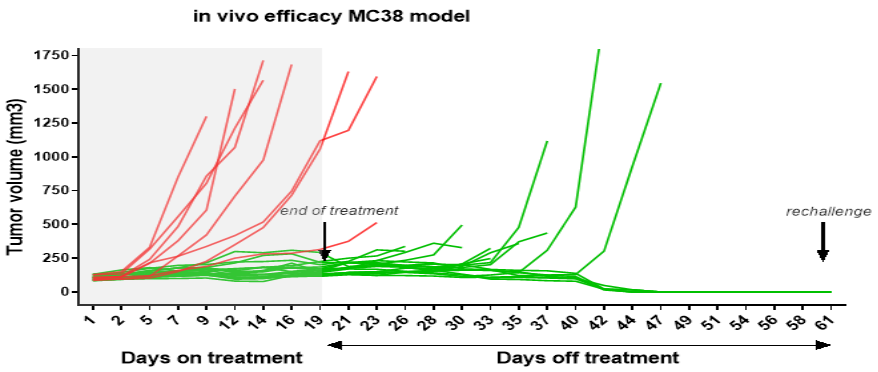
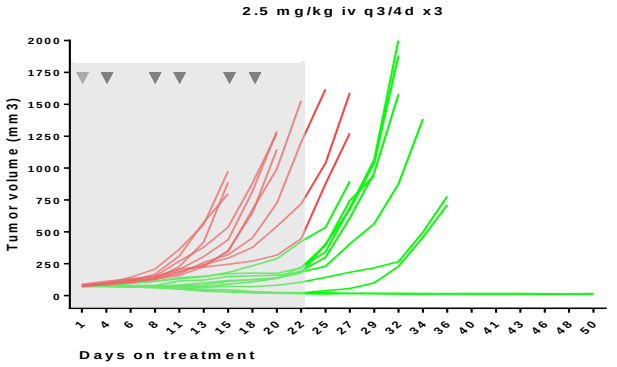
In vivo-efficacy upon *i.tu* and *i.v.* administration – MC38 mouse model

Intratumoral administration 6 µg/10 µl *i.tu.*



***i.v.* administration 2.5 mg/ kg (>MTD*)**



- Intratumoral administration demonstrates strong efficacy in MC38 model (6/19 tumor free)
- Intratumoral treatment achieves long lasting and high efficacy
- Intravenous administration of >MTD shows moderate efficacy (2/10 tumor free)

* MTD = maximum tolerated dose in explor. toxicology study was 1 mg/kg

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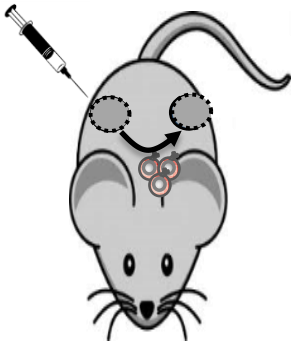
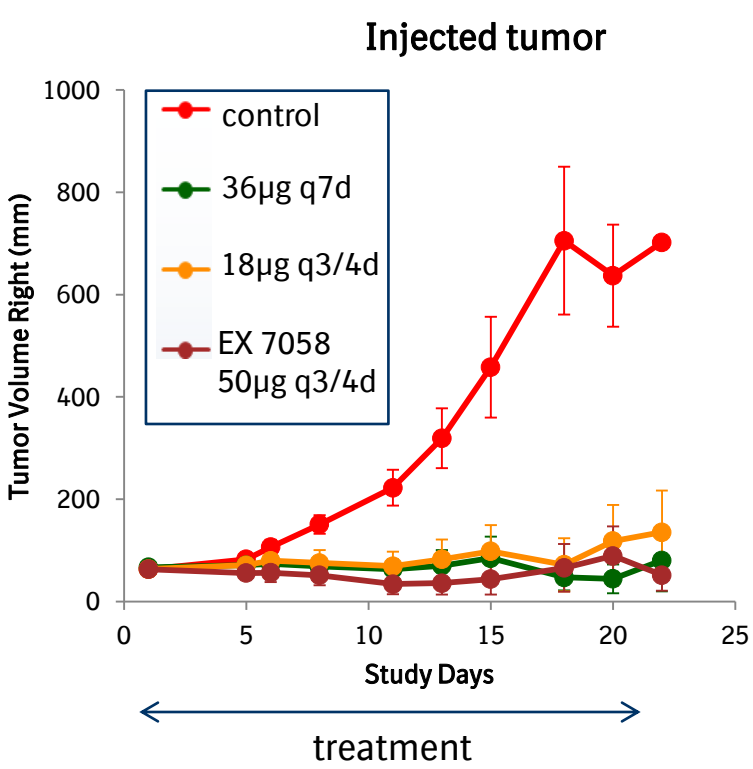
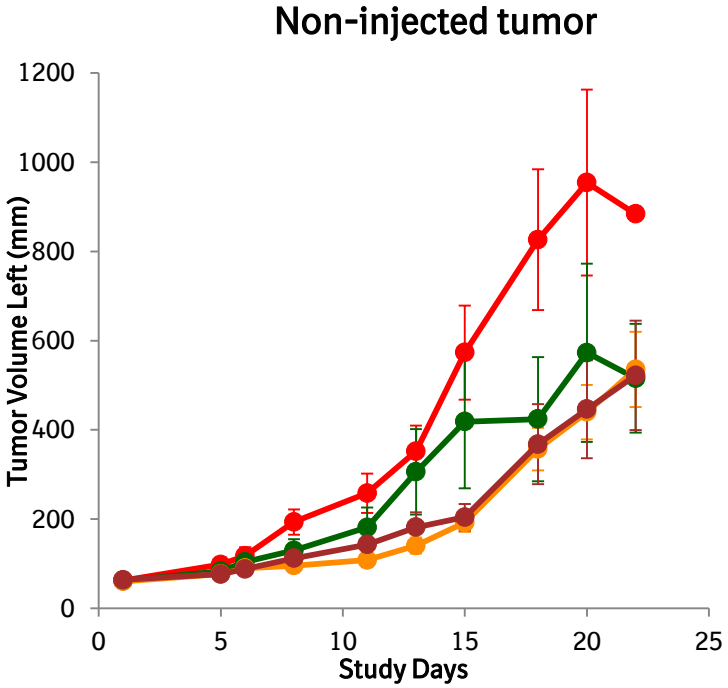
Induction of weak abscopal/anenestic effect



MC38
tumor model





STING agonist i.tu. weakly reduced tumor growth in non-injected lesion

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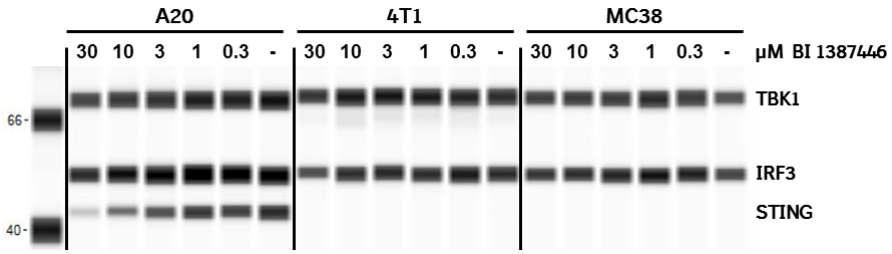
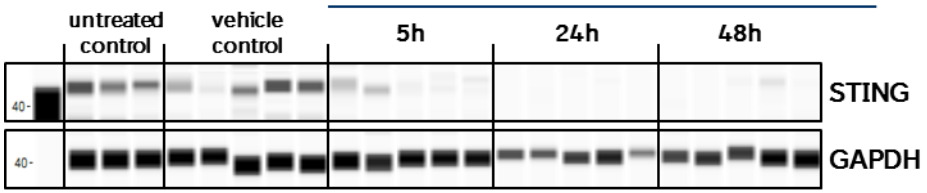
STING Degradation

- Efficacy seems to depend on surges of cytokine release (daily treatment less efficacious than intermittent treatment)

Mouse tumor cells *in vitro* – STING agonist 4 h treatment



Mouse 4T1 tumors *in vivo* – STING agonist 4 µg/10 µl *i.tu.*



→**STING degradation both *in vitro* and *in vivo* within 4-6 h upon treatment** (for *i.tu.* → injected tumor only)

→Upon wash-out, cellular STING levels are restored within 2-3 days (3-4 days *in vivo*)

→**Hypothesis for PK/PD:** ~5 h exposure in tumor > $c_{eff.}$ sufficient – long PK half-life or tumor exposure not required!

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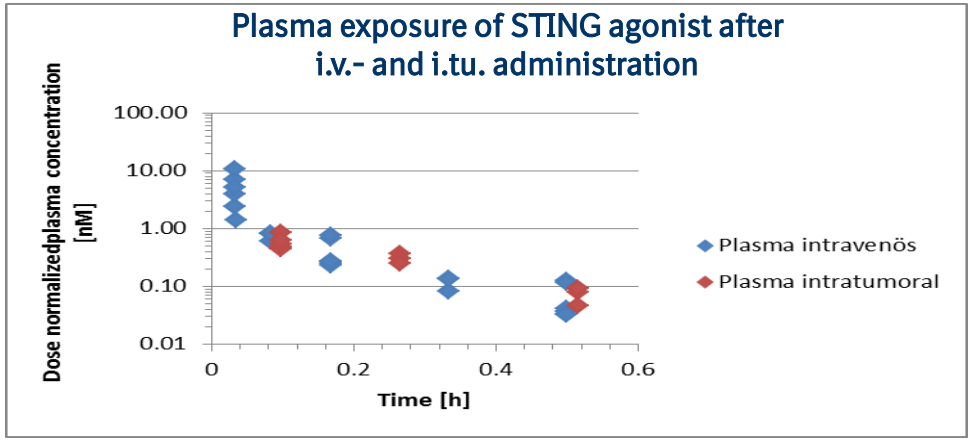
”Tumor leakage” - Meta Analysis of Mouse PK Data

- Dose-normalized exposure following intravenous and intratumoral administration comparable from early time points (0.083h = 5min) on



Intravenous dosages:
- 0.3-3 mg/kg, 25 data points
- Mean conc @0.083h 0.75±0.13 nM/(µg/kg)
- Mean conc @0.5h 0.065±0.045 nM/(µg/kg)

Intratumoral dosages:
- 2-36 µg/tumor (0.067-1.2 mg/kg), 14 data points
- Mean conc @0.083h 0.57±0.16 nM/(µg/kg)
- Mean conc @0.5h 0.073±0.023 nM/(µg/kg)

Half life (0.083 h-0.5h):
- Intravenous: 0.16h
- Intratumoral: 0.14h

- At the earliest time point investigated (0.083h = 5min), on average 50% (50±11%, range 33-70%, N=9) of the dose localized in the tumor

→ Following intratumoral administration in mice at very early time points about 50% of the dose is already distributed to the systemic circulation

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2-cycle exploratory i.v. toxicity study in mice

Study design

- Species: Mouse
- Strain: C57BL/6
- Animal nos.: 5+3 males/5+3 females per dose level (main + TK)
- Dose levels: 0, 1.0, 3.0/0.3 (3^{rd} admin males; 2^{nd} admin females) & 10.0 mg/kg
- Route: i.v.
- Schedule: Two 1 week-cycles, administration on 1^{st} and 4^{th} day of each cycle

- Study parameters as usual including cytokine measurements (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, KC/GRO, IL-10, IL-12p70, TNF-α; type 1 interferons; 4h p.adm.)

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2-cycle exploratory i.v. toxicity study in mice

Results

- Clinical signs: 10.0 mg/kg: hunched back, stilted gait, activity↓
- Decedents: 3.0 mg/kg: 1 male (D6); 10 mg/kg: 2 males/4 females (D5)
- Body weights: Dose-dependent temporary decreases, at lower doses some evidence for adaptation
- Clin. chemistry: AST/ALT/glucose (m)/t-chol/glob↑
  trig/BUN (m)/glucose (f)/alb/A:G↓
- Hematology: WBC↓, Ly↓, PLT↓, PMN↑
- Cytokines: Increases in IL-5 (+), IL-6 (++++), KC/GRO (+++), IL-10 (+++), IL-12p70 (+++), TNF-α (+++)

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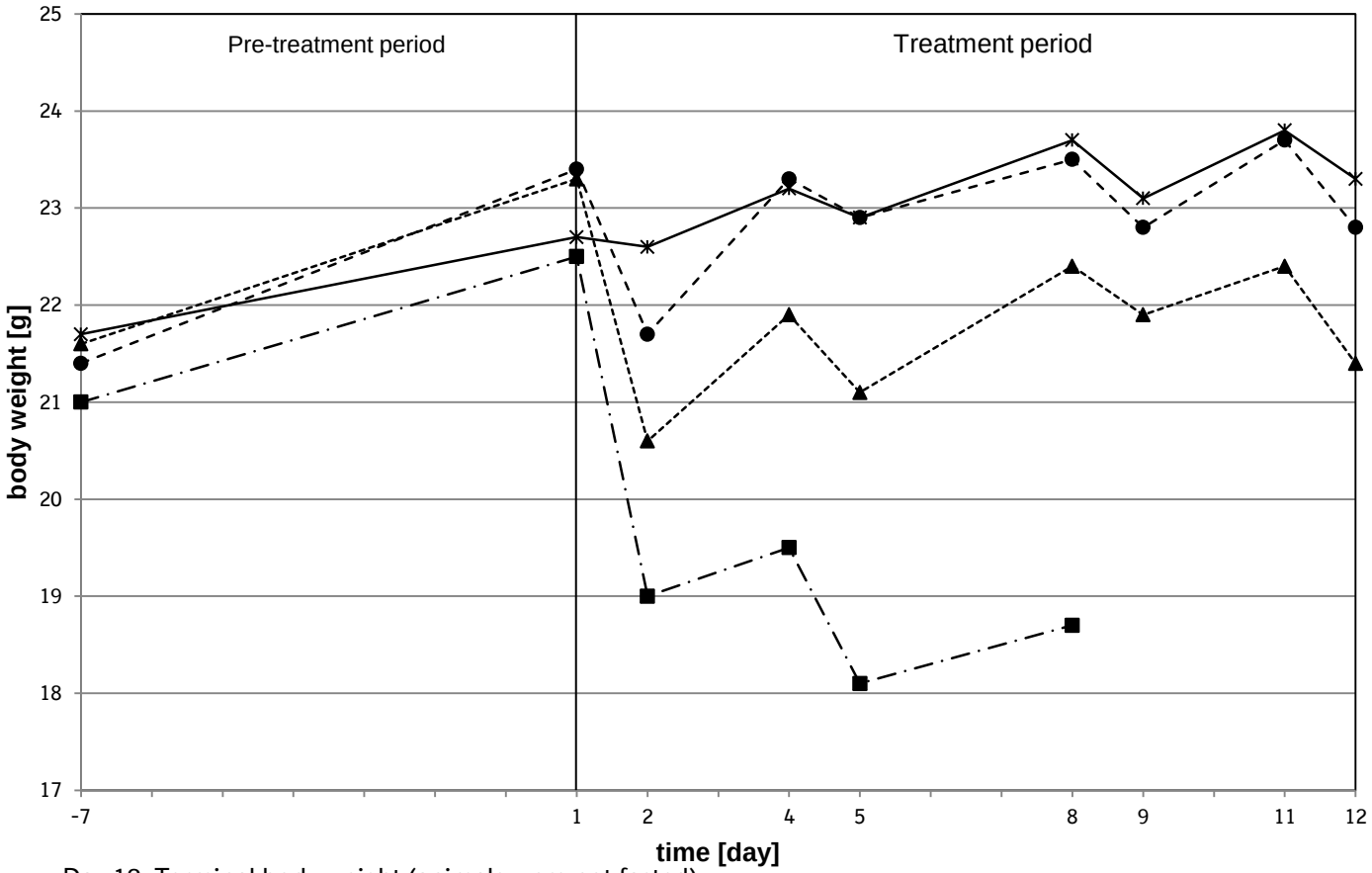
2-cycle exploratory i.v. toxicity study in mice

Exploratory 2-week intravenous (bolus) toxicity study in C57BL/6 mice
Mean absolute body weight [g] - males



Day 12: Terminal body weight (animals were not fasted).
Due to high mortality in Group 4, certain toxicokinetic animals are included.

0 mg/kg (Control; Group 1) — 1 mg/kg… — 3 mg/kg on Day 1, 4… — 10 mg/kg…

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2-cycle exploratory i.v. toxicity study in mice – IL-6

Male		IL-6 [pg/mL]	Male		IL-6 [pg/mL]	Male		IL-6 [pg/mL]	Male		IL-6 [pg/mL]
Dose Group	Animal Number	Day -7	Dose Group	Animal Number	Day 11 4h	Dose Group	Animal Number	Day 11 4h	Dose Group	Animal Number	Day 11 4h / Day 8 #
Group 1 / M	101	6,94	Group 2 / M	K206	837,24	Group 3 / M	K306	1708,19	Group 4 / M	K406	N/S
0 mg/kg	102	5,24	1 mg/kg	K207	418,77	3 mg/kg	K307	457,59	10 mg/kg	K407	N/S
	103	13,41		K208	255,67	0.3 mg/kg*	K308	1571,40		K408	N/S
				K209	355,54		K309	3285,08		K409	N/S
				K210	3507,76		K310	337,45		K410	N/S
				K211	>ULOQ		K311	N/S		K411	N/S
										402[L]	N/S

Female		IL-6 [pg/mL]	Female		IL-6 [pg/mL]	Female		IL-6 [pg/mL]	Female		IL-6 [pg/mL]
Dose Group	Animal Number	Day -7	Dose Group	Animal Number	Day 11 4h	Dose Group	Animal Number	Day 11 4h	Dose Group	Animal Number	Day 11 4h / Day 5 +
Group 1 / F	151	5,74	Group 2 / F	K256	>ULOQ	Group 3 / F	K356	1315,85	Group 4 / F	K456	N/S
0 mg/kg	152	10,76	1 mg/kg	K257	>ULOQ	3 mg/kg	K357	1896,88	10 mg/kg	K457	N/S
	153	6,12		K258	>ULOQ	0.3 mg/kg*	K358	650,18		K458	N/S
				K259	8050,98		K359	679,76		K459	N/S
				K260	2109,65		K360	2332,28		K460	N/S
				K261	>ULOQ		K361	1507,76		K461	N/S
										451[L]	204,47
										K461[L]	73,25

* from Day 8

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2-cycle exploratory i.v. toxicity study in mice

Results

- Organ weights: 3.0/10.0 mg/kg: Testes/thymus/(liver)↓, spleen(↑)
- Gross pathology: Lnn./thymus/prostate/seminal ves. reduced in size
- Histopathology: *Testes:* degeneration spermatocytes, atrophy seminiferous tubules; *epididymides:* debris; *prostate/seminal vesicles:* secretory activity↓; *GI tract:* debris in crypts; *thymus:* cellular depletion; *lnn/spleen:* cellular depletion (T-cell areas > B-cell areas); *femur:* cellular depletion, fibrosis; *liver:* acute necrosis; *injection site:* ++ inflammation

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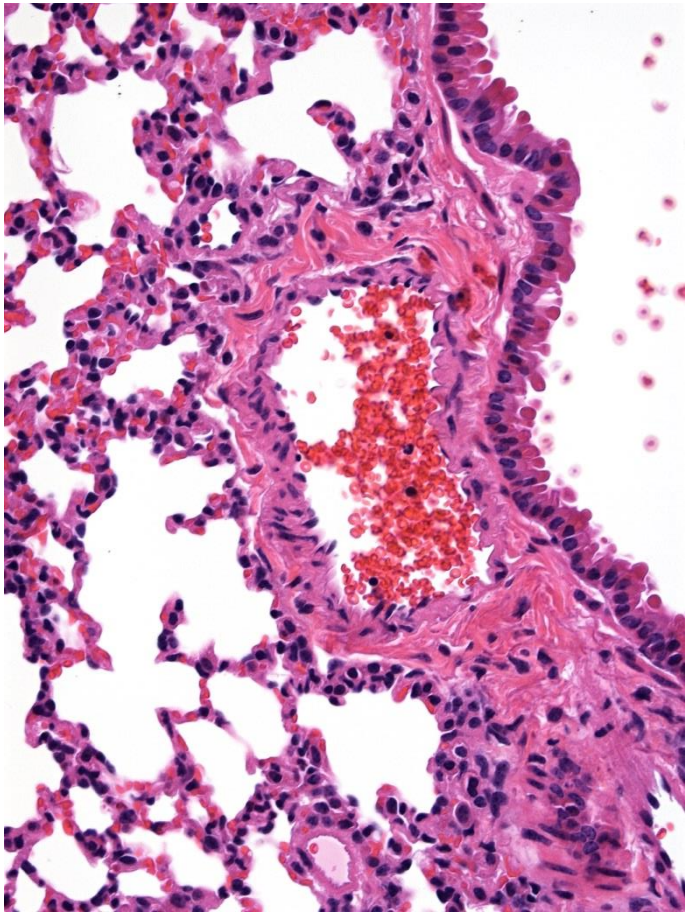
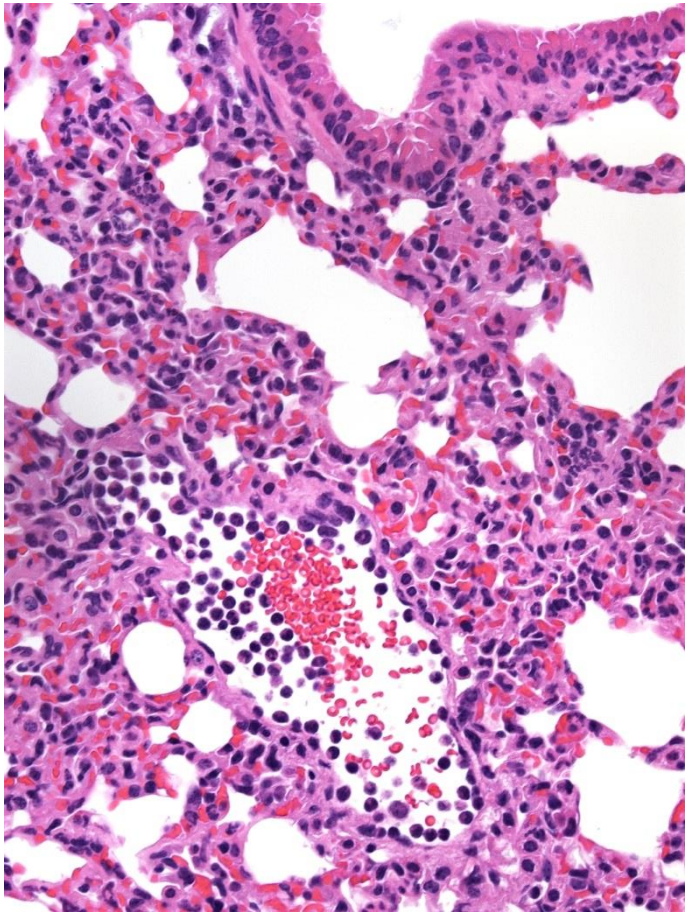
2-cycle exploratory i.v. toxicity study in mice

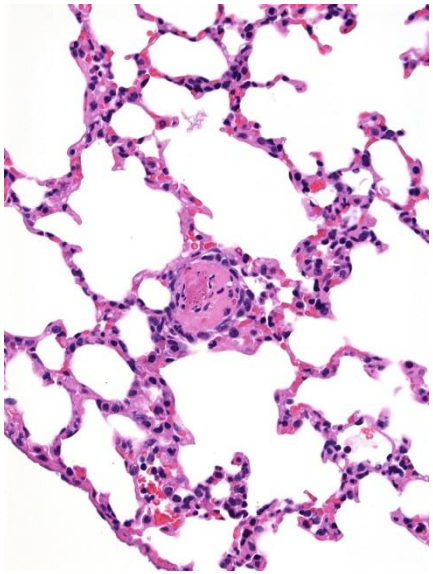
Histopathological findings – lung



G4 – 102 – HE – 40x



G4 – 405 – HE – 40x



G3 – 301 – HE – 40x

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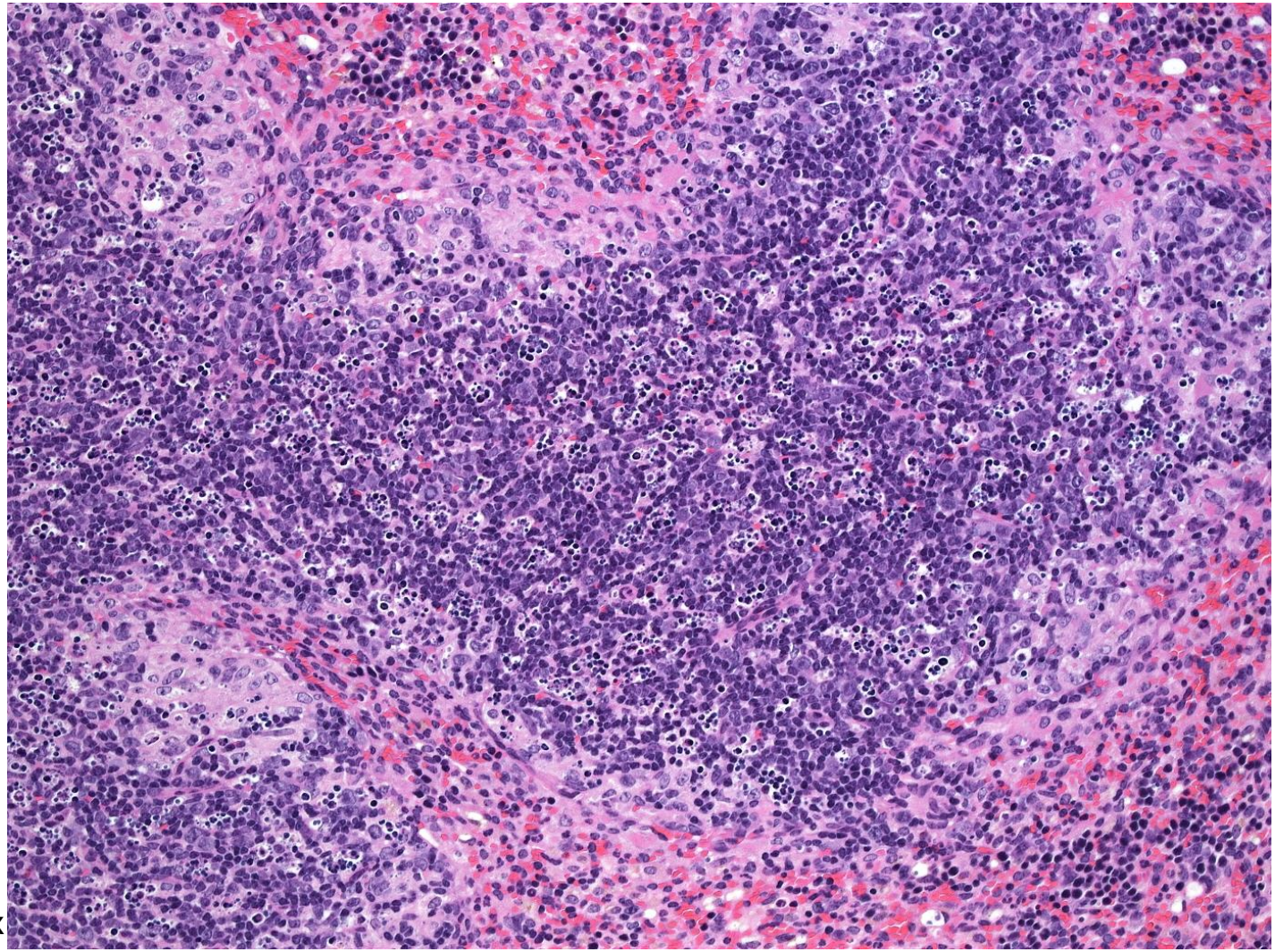
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings – spleen



G4 – K457 – HE – 20x

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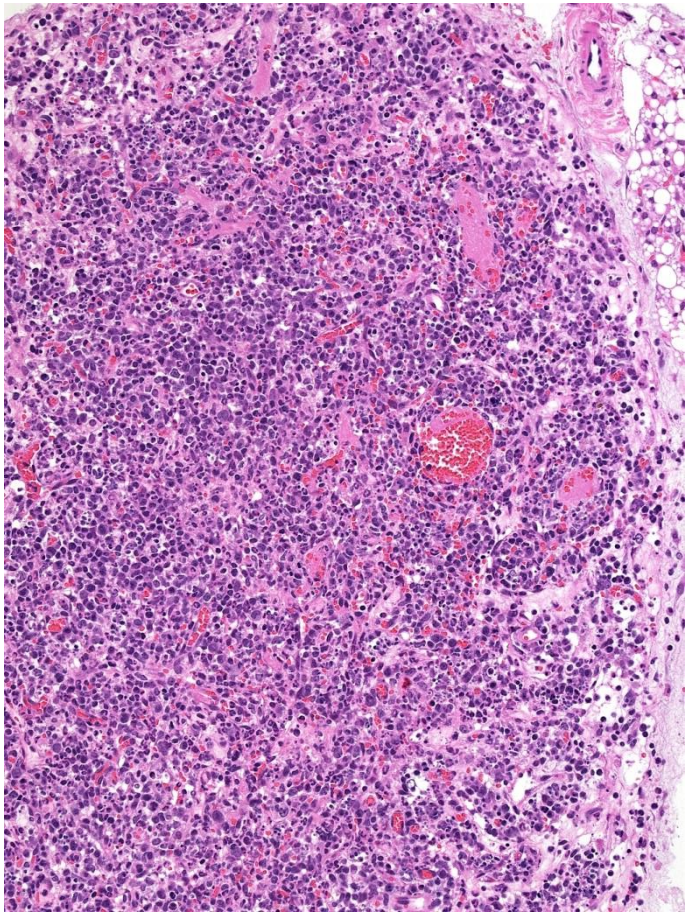
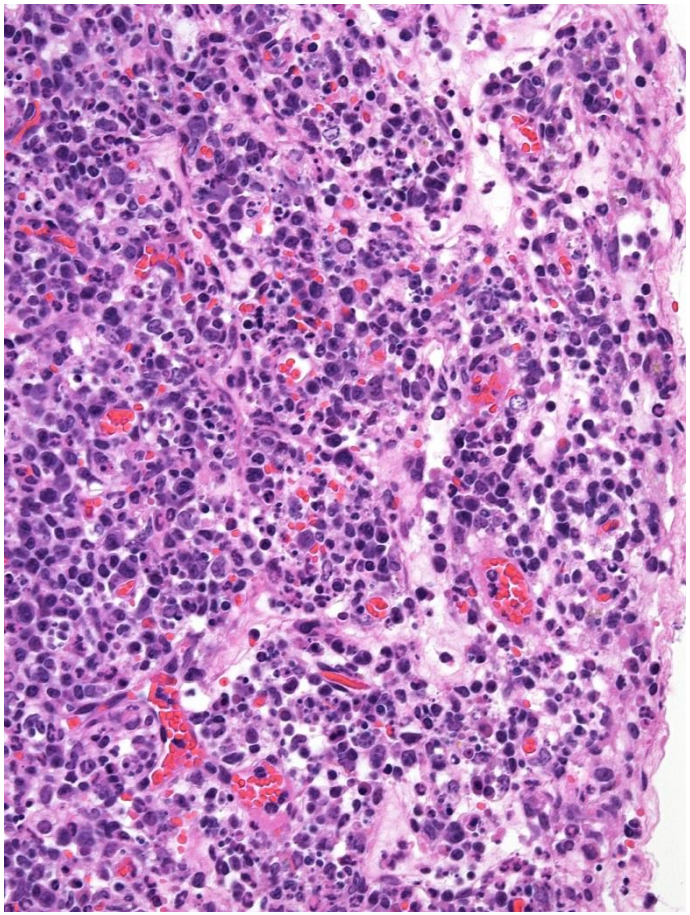
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings – iliac lymph node



G4 – 402 – HE – 20x



G4 – 402 – HE – 40x

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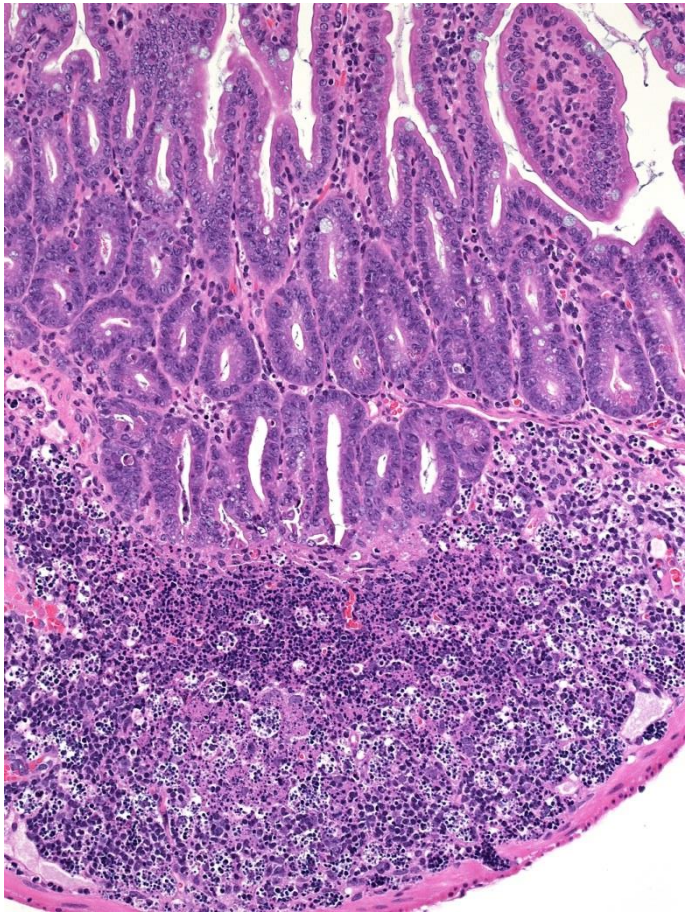
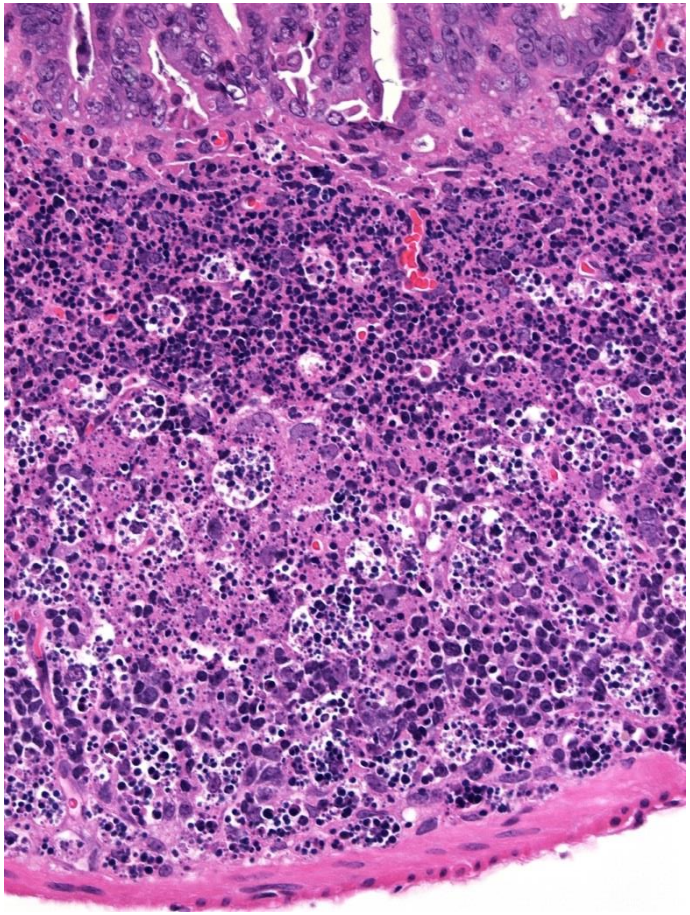
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings – jejunum/Peyer's patch



G4 – K458 – HE – 20x



G4 – K458 – HE – 40x

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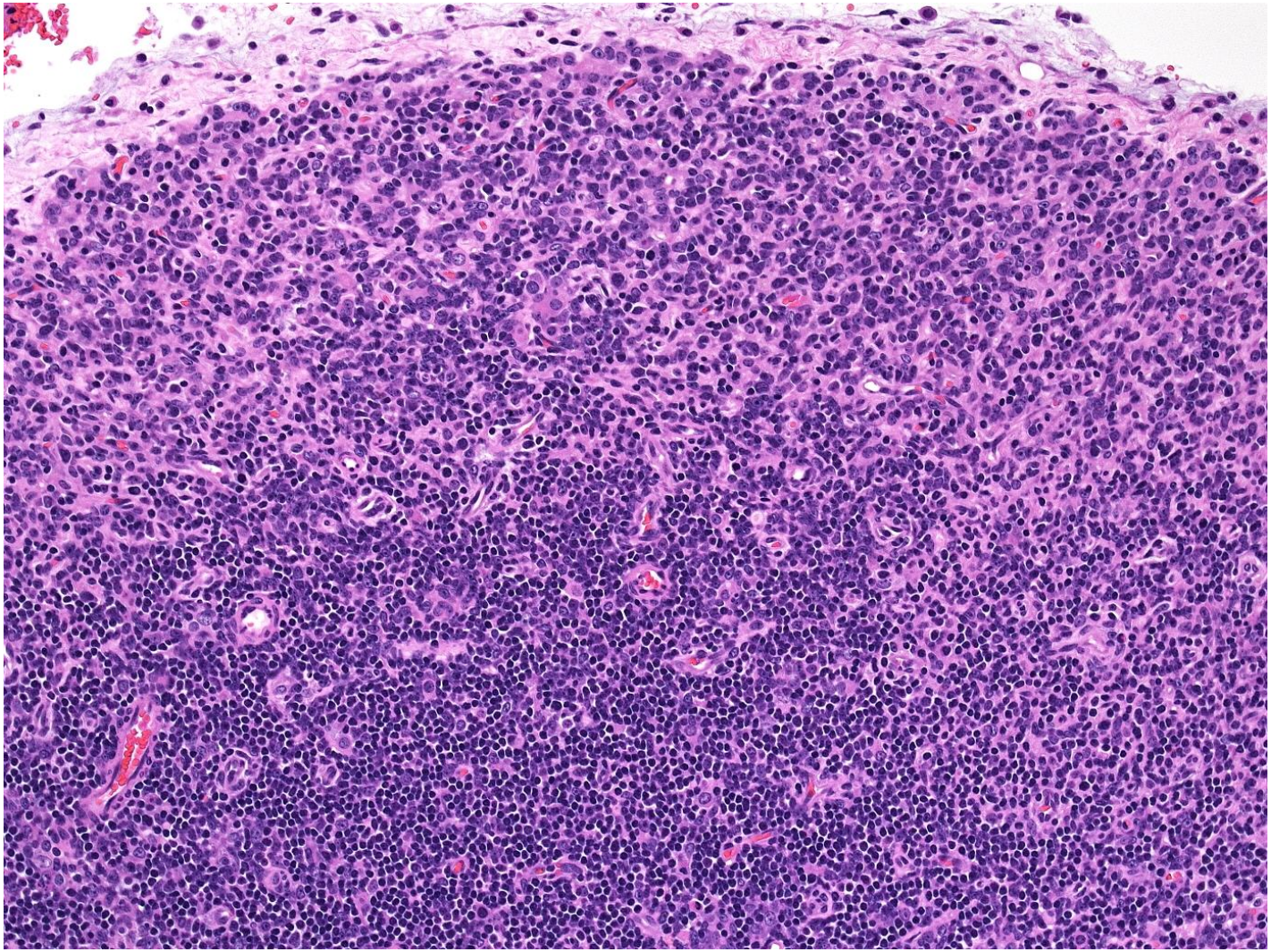
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings – thymus



G4 – 402
– HE – 20x

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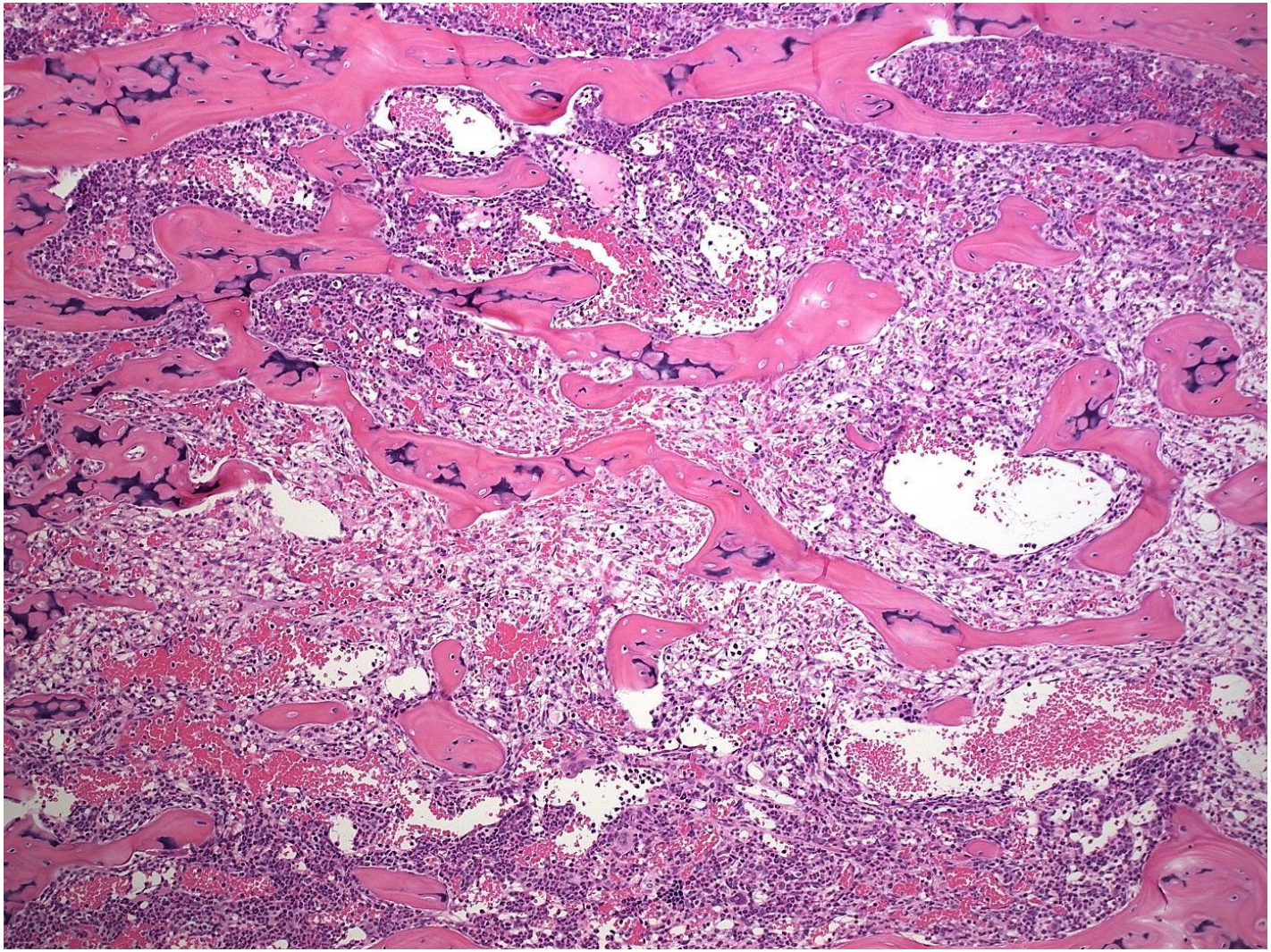
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings - femur



G4 – 402 – HE – 10x

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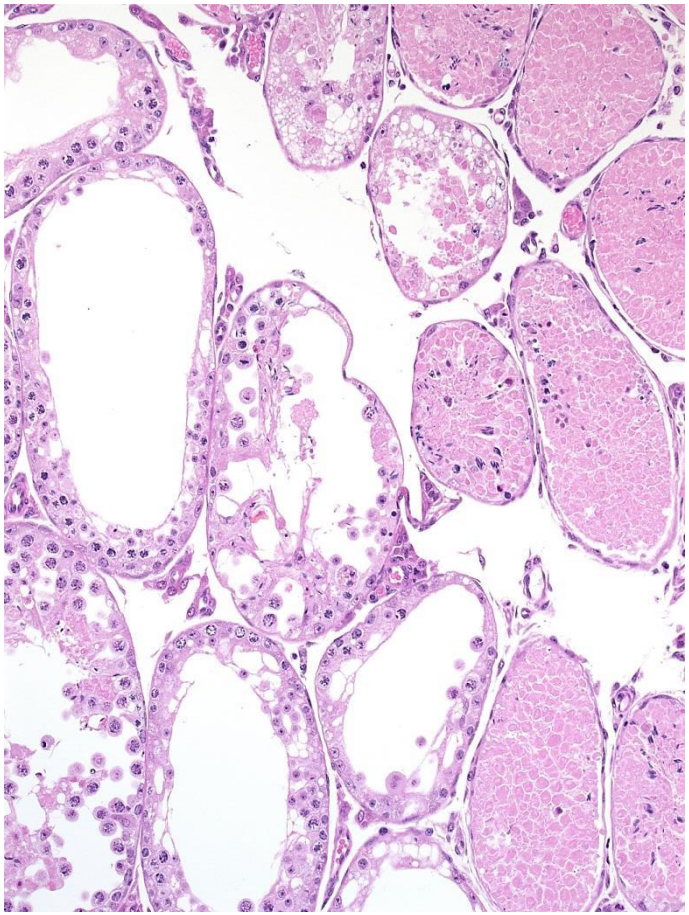
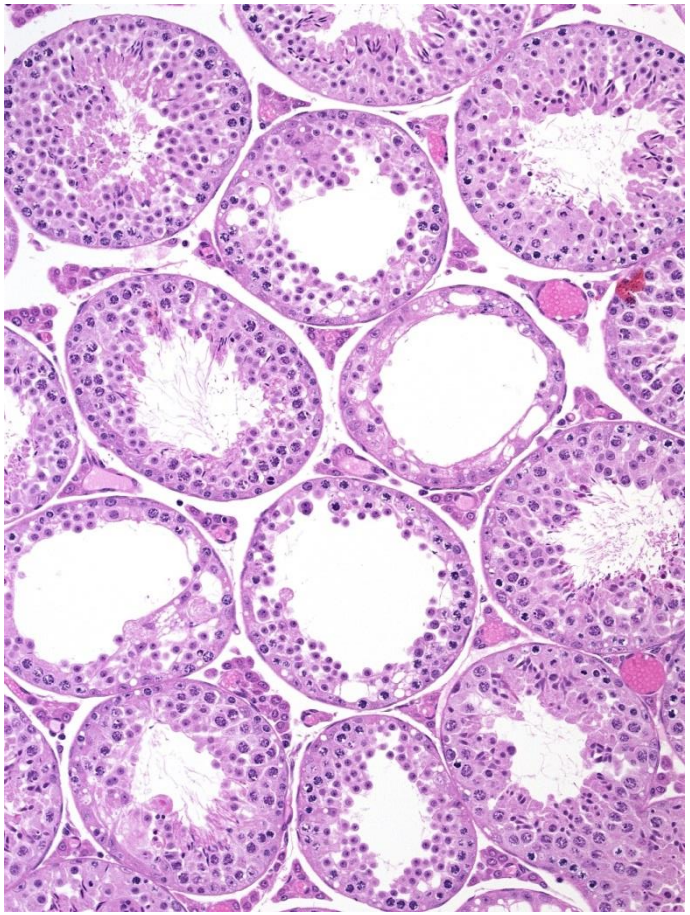
2-cycle exploratory i.v. toxicity study in mice

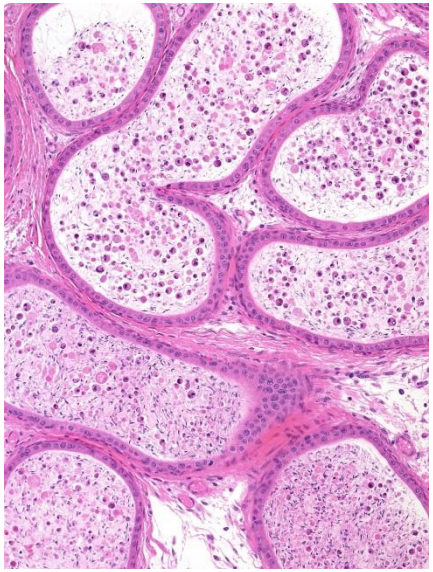
Histopathological findings – testes/epididymides



G4 – 405 – HE – 20x



G4 – 402 – HE – 20x



G4 – 402 – HE – 20x

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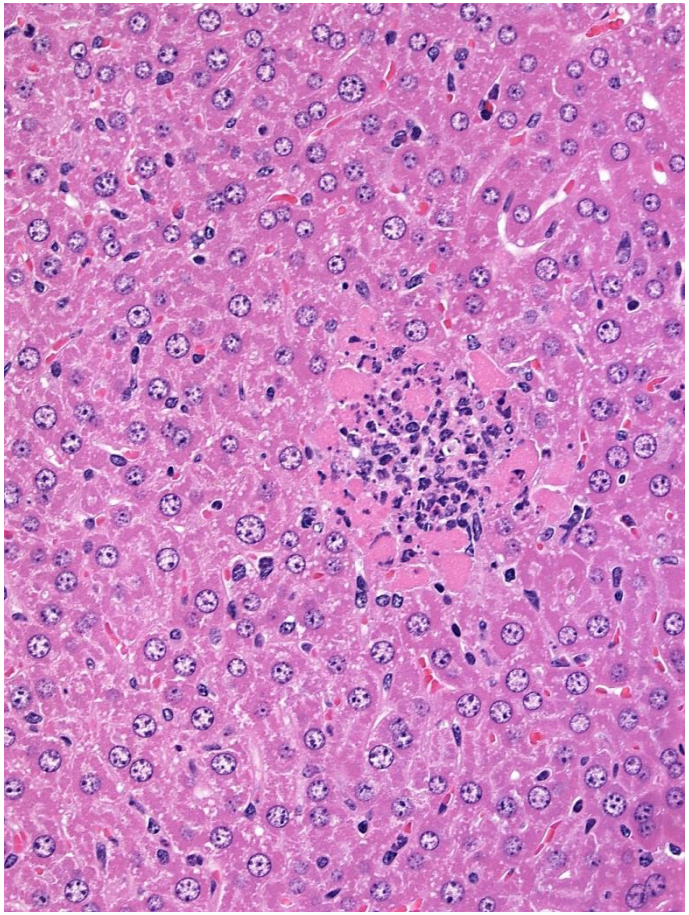
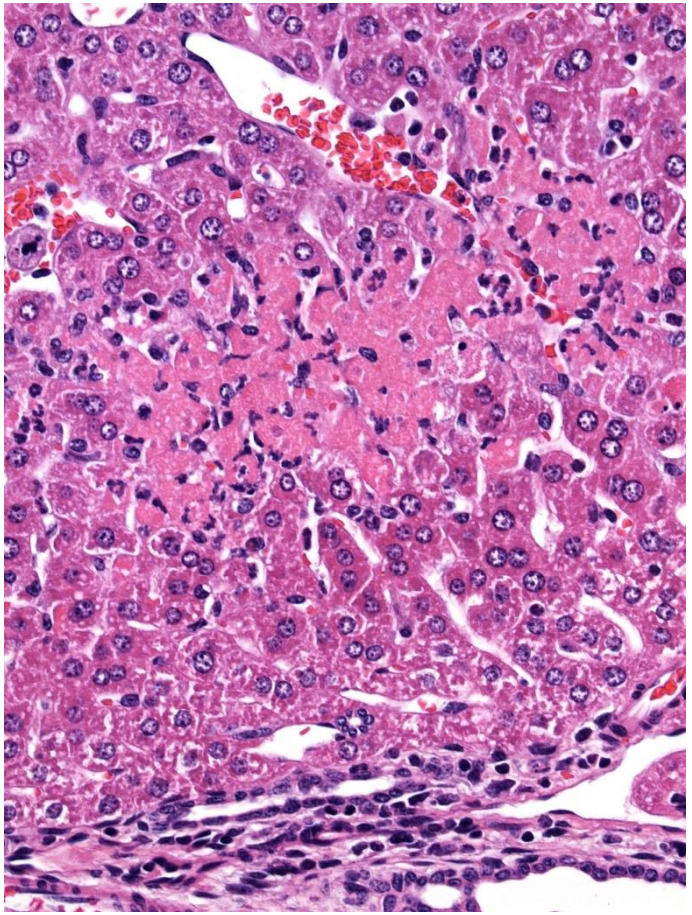
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings - liver



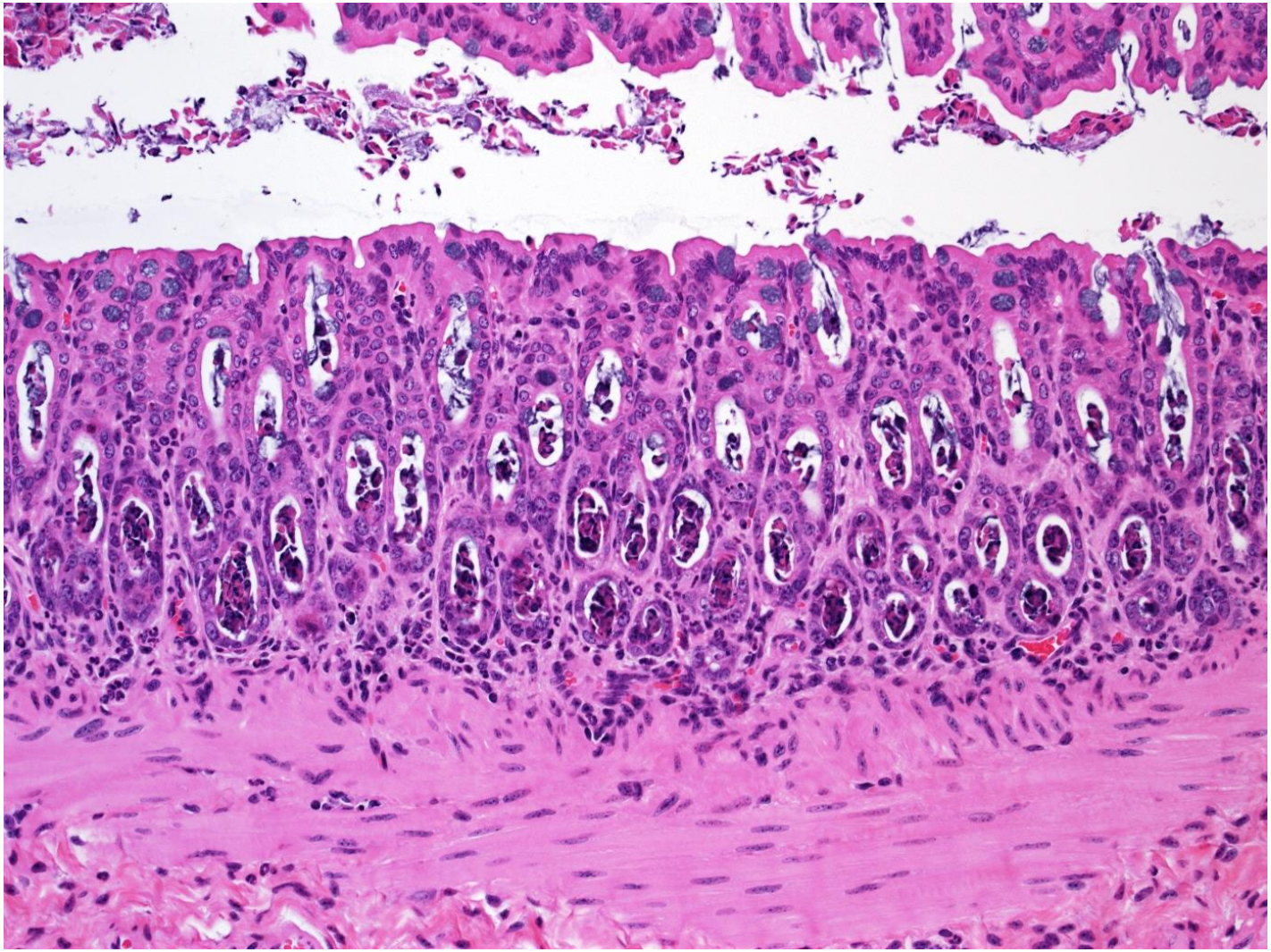
G4 – K456 – HE – 40x



G4 – K459 – HE – 40x

STING Agonists
2-cycle exploratory i.v. toxicity study in mice

Histopathological findings - rectum



G4 – K456 – HE – 20x

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4-cycle toxicity study in mice (ongoing)

Study design

Treatment:	4 cycles with 2 i.v. administrations on Days 1 and 4 in each cycle
Dose levels:	0.3, 1.0 and 3.0 mg/kg, i.v.

Preliminary results

Clinical chemistry:	AST↑, ALT↑, Triglyc↓, Gluc↓, BUN ↓, Alb↓, Glob↑, A/G↓
Hematology:	WBC↓, Neu↑, Eos↓, Ly↓, LUC↑, Hb↓, RBC↓, PCV↓, Reti↓, RDW↑, Plt↓
Organ weights:	Spleen↑, lymph nodes↑
Gross pathology:	*Spleen, lymph nodes:* Dose-dependent increase in size

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Escalating dose/DRF study in Cynomolgus monkeys

Route: Intravenous (bolus); 5 mL/kg

Dosing: **Esc. Phase:** maximum of seven administrations once weekly (Days 1, 8, 15, 22, 29, 43 and 87)
Anti-IL-6-antibody administered once about 24h prior to STING agonist at the end of the escalating dose phase
TK sampling (all dosing days): 2min – 15min – 30min – 1 hr – 2 hrs – 4 hrs

DRF Phase: four administrations twice weekly over a period of 14 days (dosed on Days 1, 4, 8 and 11)
TK sampling (Day 1 and 11): predose – 2min – 15min – 30min – 1 hr – 2 hrs – 4 hrs

Phase	Group	Animal numbers	
		males	females
Esc. Phase	1	501	507
DRF Phase	2	**502****, *(513)*	*(508, 514)*
	3A	**503***, 504	509, 510
	3B	504	509, 510
	4	505, 506	511, 512

*** Due to premature death of No. 503M on Day 5, dosing was ceased in Group 3A following Day 4 dose. Dosing was restarted for all animals (Group 3B).**

**** Unexpected death of No. 502M after first dosing**

Phase	Group	Dose [mg/kg]	Day	AUC(0-4h)/dose [(nmol·h/L)/(mg/kg)]	
				male	female
ESC PHASE	1	0.1	1	1000	1270
		0.3	8	1150	1620
		1	15	1120	1480
		2	22	1300	1750
		2	29	851	1680
		3	43	1500	1630
		3 (RoAct.)	87	1310	2090
DRF PHASE	2	0.6	1	1190	---
	3A	0.6	1	1010	1080
	3B	0.6	1	1420	1170
			11	1260	906
	4	0.3	1	1120	982
			11	1090	1200

STING Agonists

Escalating dose/DRF study in Cynomolgus monkeys - IL-6 release

Cytokine	IL-6 [pg/mL]															
Dosage	0.3 mg/kg (M505, M506, F511, F512); 0.6 mg/kg (M502, M504, F509, F510)															
	Day 1								*Day 4*							
Animal	M 502	M 504	M 505	M 506	F 509	F 510	F 511	F 512	M 502	M 504	M 505	M 506	F 509	F 510	F 511	F 512
Predose	<0.34	0,4	0,7	0,5	1	1	0,4	1	-	2	9	0,9	0,5	8	0,5	1
4 h	>1530	339	300	276	98	344	26	114	-	46	14	8	9	534	44	18
24 h	-	26	587	8	3	397	4	9	-	2	3	5	1	24	5	2
	Day 8								*Day 11*							
Predose	-	<0.34	0,4	0,5	0,5	0,6	0,6	0,8	-	<0.34	0,4	1	0,4	0,8	0,9	0,7
4 h	-	8	7	7	4	46	2	17	-	39	29	27	6	62	71	28
24 h	-	1	4	3	1	17	2	6	-	2	2	5	5	3	4	1

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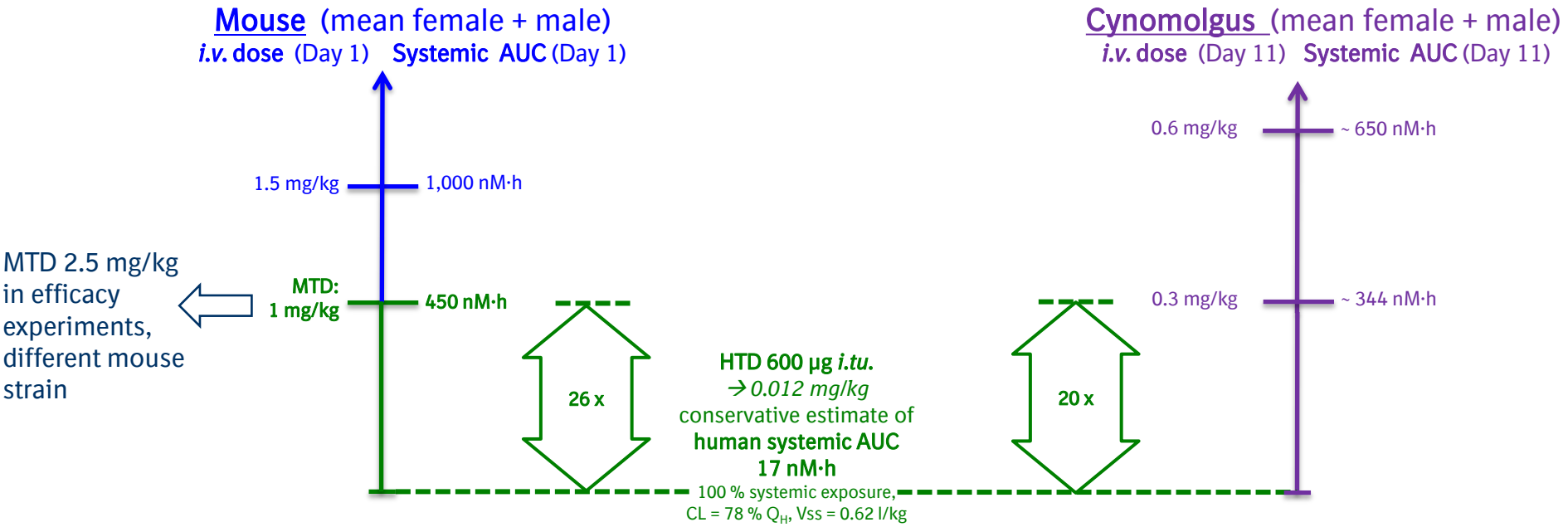
Escalating dose/DRF study (i.v.) in Cynomolgus monkeys - Summary

- Single administration of salt form A of STING agonist up to 3 mg/kg well tolerated (n=2)
- Single administration of salt form B not tolerated at 0.6 mg/kg by one animal due to individually high cytokine release
- 2-cycle treatment with salt form B (Day 1 and Day 4 per week) up to 0.3 mg/kg well tolerated
- Cytokine release after first administration higher than after consecutive administrations
- No pertinent changes in study parameters such as clinical pathology or histopathology
- Administration of anti-IL-6-antibody 24 h before single administration of STING agonist not tolerated due to severely enhanced cytokine release

STING Agonists

Estimated exposure multiples



→ Substantial therapeutic window expected for HTD 600 µg *i.tu.*

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Use for anti-cancer treatment

Translation into the clinics?

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Use for anti-cancer treatment – open questions

Route:	Intratumoral or others (i.v., s.c.)?
Volume:	Small or large?
Concentration:	Low or high?
Schedule:	Standard every 3-week regimen/cycles as in systemic chemotherapy trials or schedules based on immunisation schemes?
	Alone or in combination (e.g., irradiation, chemotherapy, check point inhibitors)?

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Use for anti-cancer treatment – open questions

- The development of non-clinical models that can predict toxicities of immune checkpoint inhibitors and stimulators in patients is an extremely important and timely issue for the cancer community. There are 50 agents targeting PD-1 or PD-L1 in clinical development, and more than 1100 trials combining anti-PD-1/L1 agents with other therapies, yet retrospective analyses indicate that animal toxicity for many of these agents is minimal and does not predict adverse effects in patients. In addition, the recent observation of worse overall survival in two randomized trials that evaluated anti-PD-1/L1 agents in combination with immunomodulatory drugs underscores the need for cross-sector collaboration in this area. With advances in nonclinical models to study the pharmacodynamics of immune checkpoint inhibitors and stimulators, the question remains whether any of these models could be adapted to assess the safety of immuno-oncology products.

FDA-AACR Workshop on Non-clinical Models for Safety Assessment of Immunooncology Products
(https://www.aacr.org/AdvocacyPolicy/GovernmentAffairs/Pages/preclinical-modeling-workshop.aspx)

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Use for anti-cancer treatment – open questions

- Also, the every 2-week and every 3-week regimen/cycles inherited from systemic chemotherapy trials as a strategy for managing the cyclical toxicity of cytotoxic agents are not relevant for immunostimulatory agents.

Thus, HIT-IT trials with innovative regimen and schedule designs are to be encouraged.

Marabelle et al. 2018 (https://doi.org/10.1093/annonc/mdy423)

Thanks for your attention!!