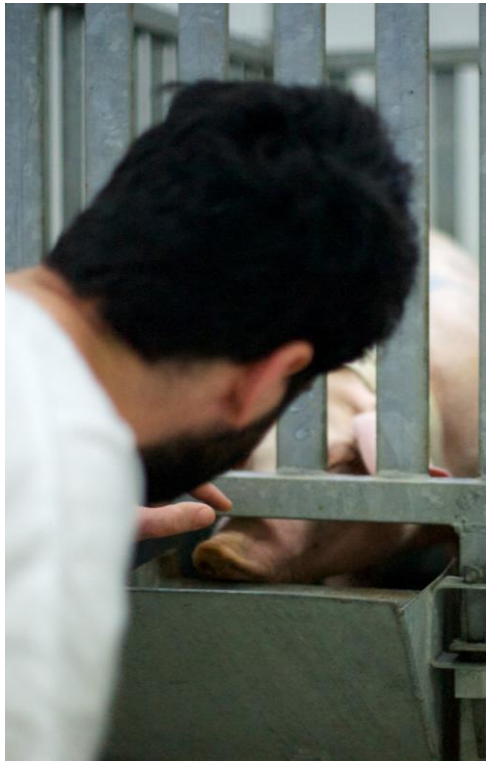
CHUR – INRAE

WP3: Orthotopic liver transplantation in pigs



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WP3 Reminder

Aim: effect of aGalCer-CD1d NP on rejection

- 2 groups:
  - Treated 10 tx 10 doses 3 weeks
  - Control 10 tx no immunosuppression

follow-up: 90 days

- 2 tasks:
  - T3.1 therapeutic effects: T-cell mediated rejection
  - T3.2 pharmacodynamic effects: presence of TR1-like liver resident iNKT

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WP3 Progress

NP batches	1	2	3
Doses	30	30	tbd
Dosage	12 mg	12 mg	tbd

Surgeries	Completed	Planned	Remaining
agalcer	4	3	3
control	3	3	4

Experiment	Group	Date	Doses used	Follow-up
1	control	March	-	complete
2	agalcer	March	8	complete
3	control	March	-	complete
4	agalcer	March	10	complete
5	control	May	-	complete
6	agalcer	June	2	complete
7	agalcer	June		in progress

- We faced delays due to lack of analgesic supply (buprenorphine) that is now resolved
- A second batch of nanoparticles will be shipped one June 17

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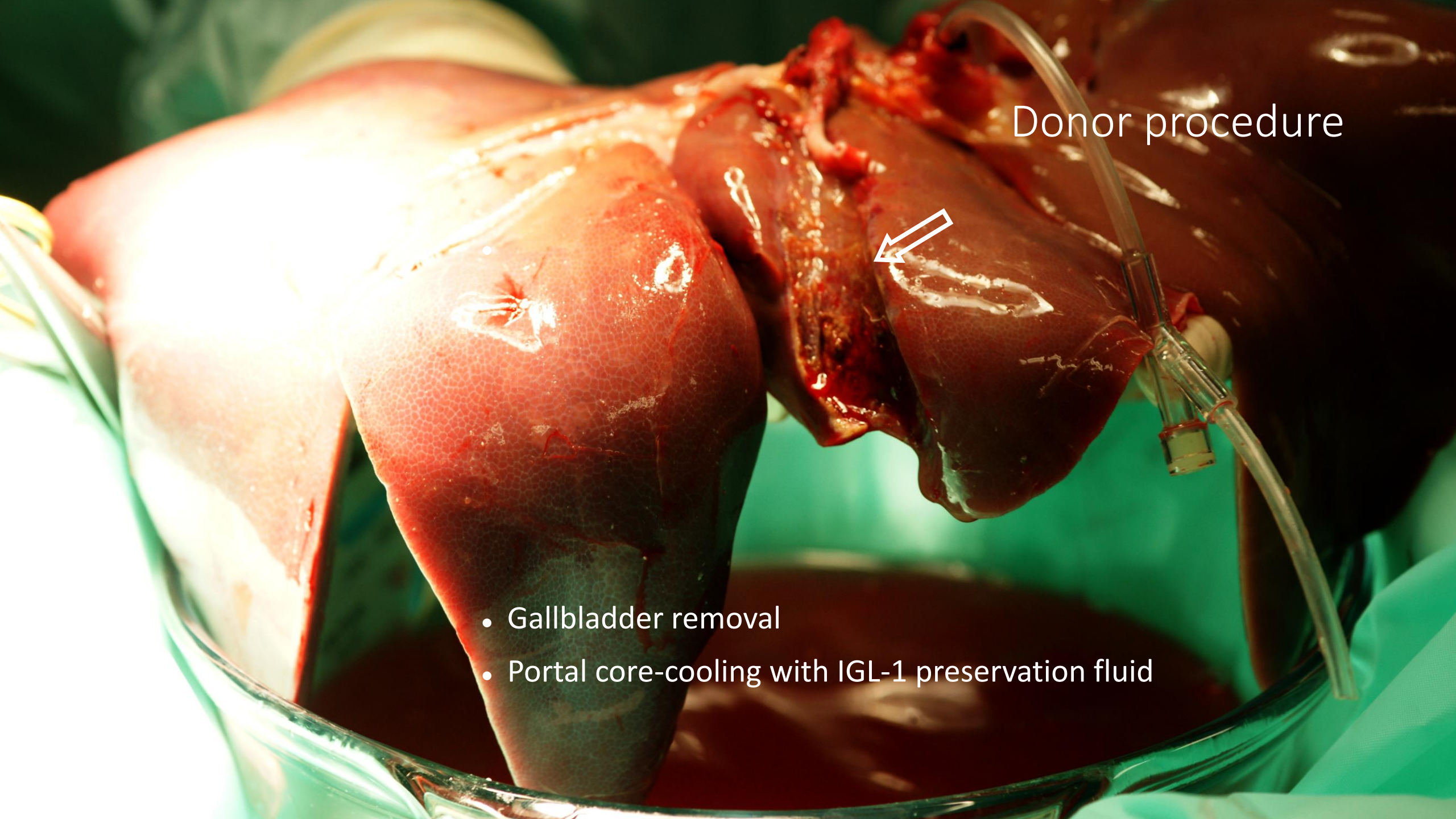
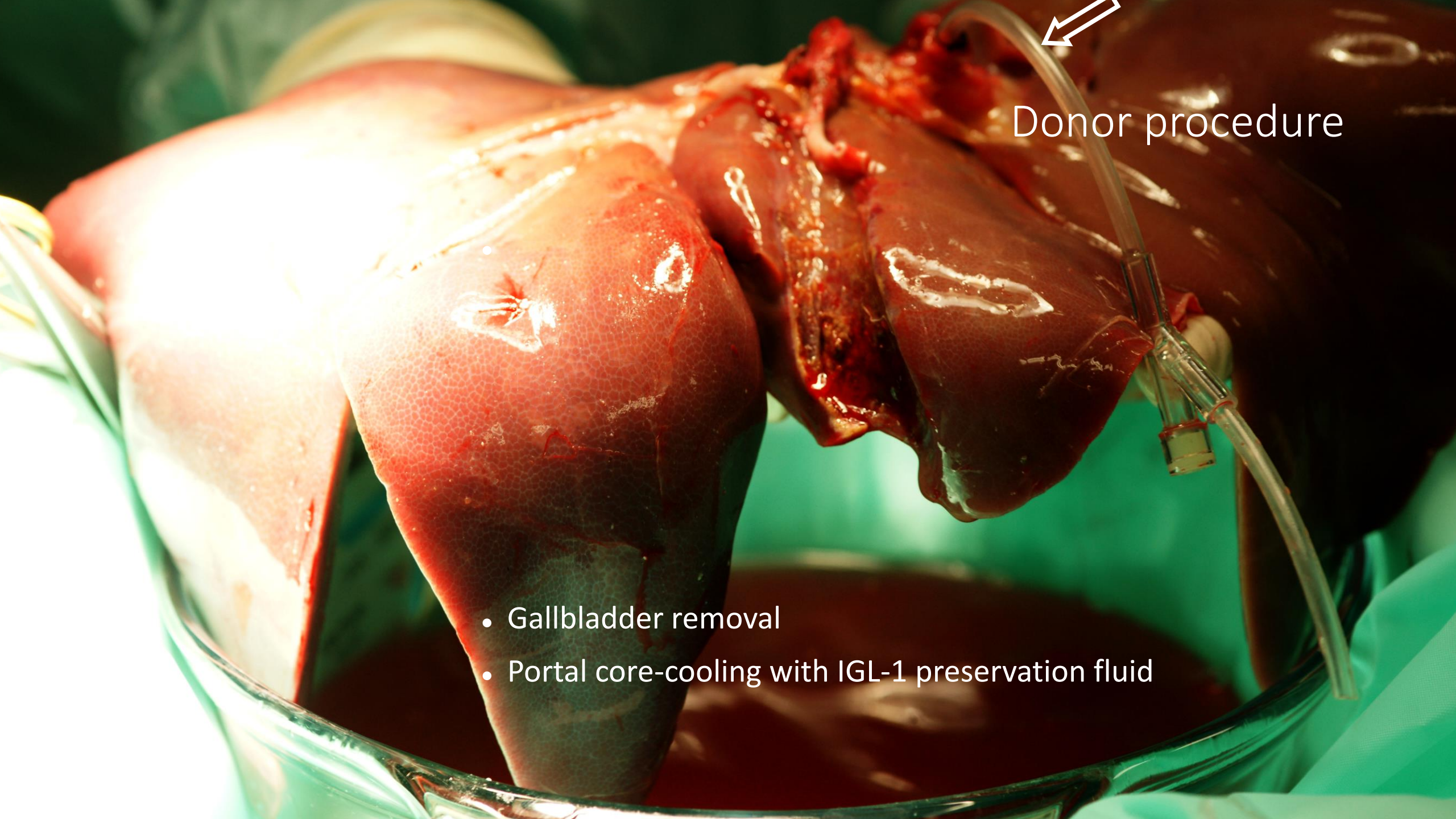
Donor procedure



- Gallbladder removal
- Portal core-cooling with IGL-1 preservation fluid



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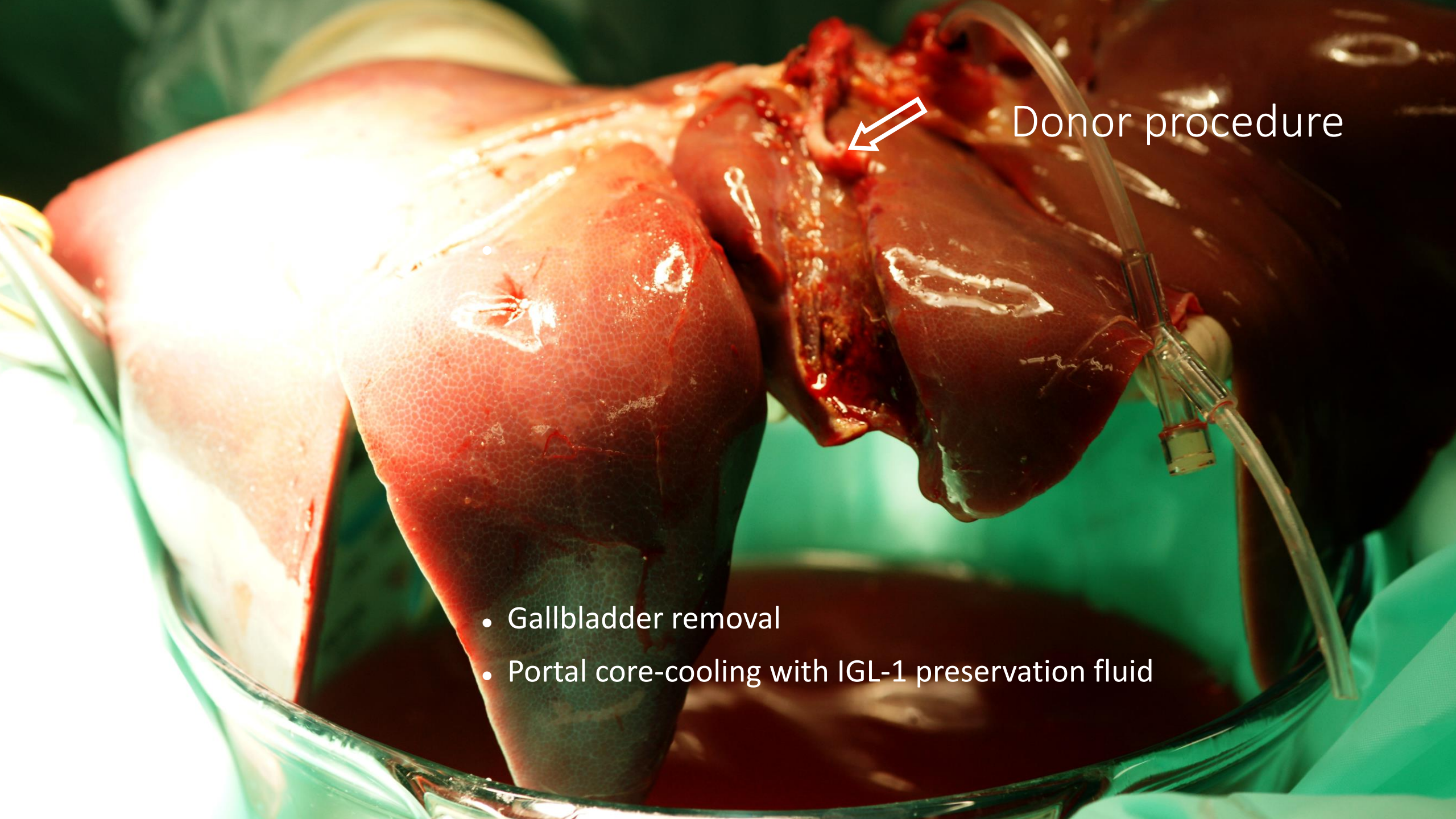

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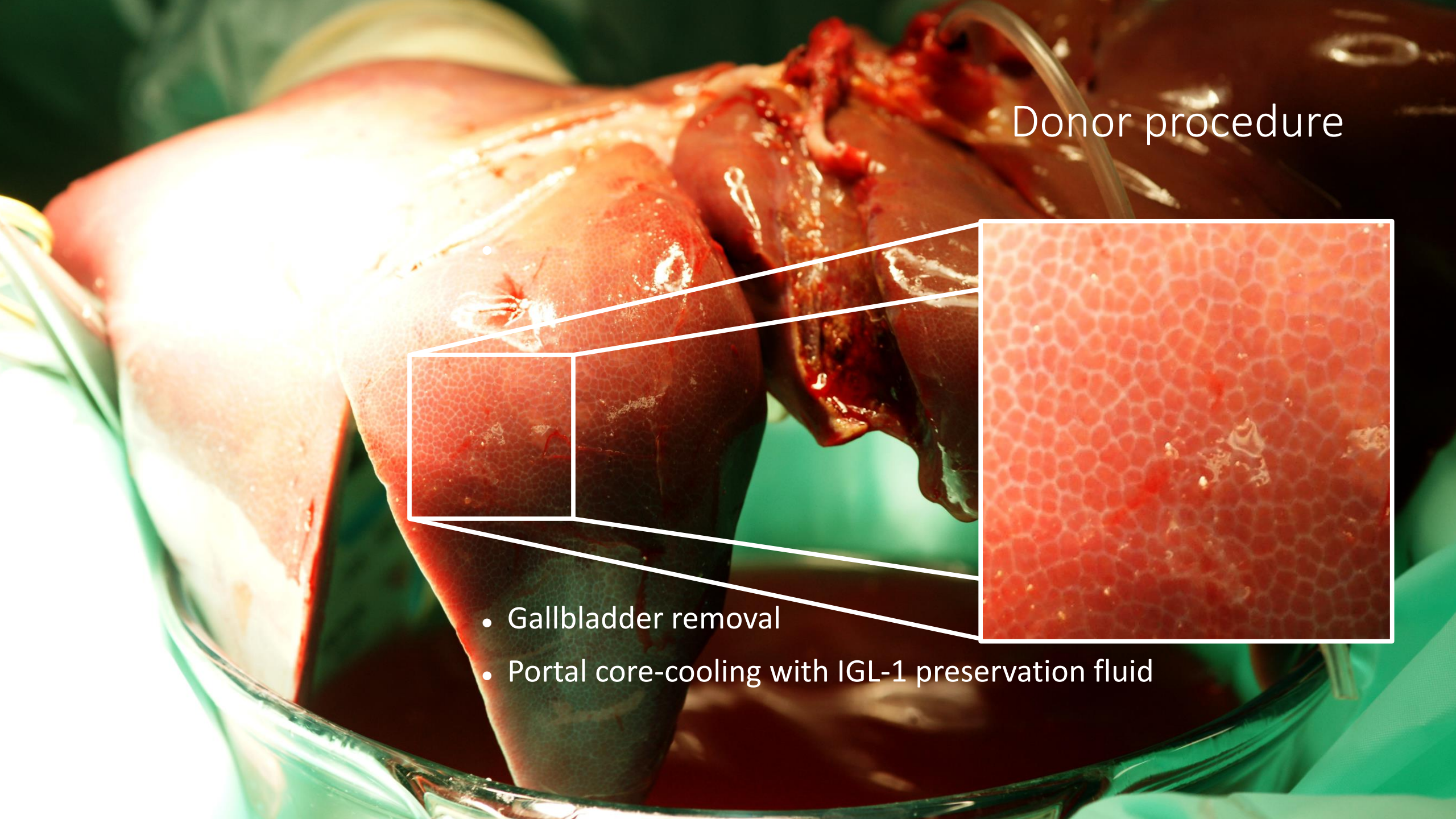

- Gallbladder removal
- Portal core-cooling with IGL-1 preservation fluid

Recipient procedure

- Standardised orthotopic liver transplantation procedure
- 3 phases:
  - Liver removal
  - Anhepatic phase
  - Reperfusion
- Passive veno-venous bypass during the anhepatic phase

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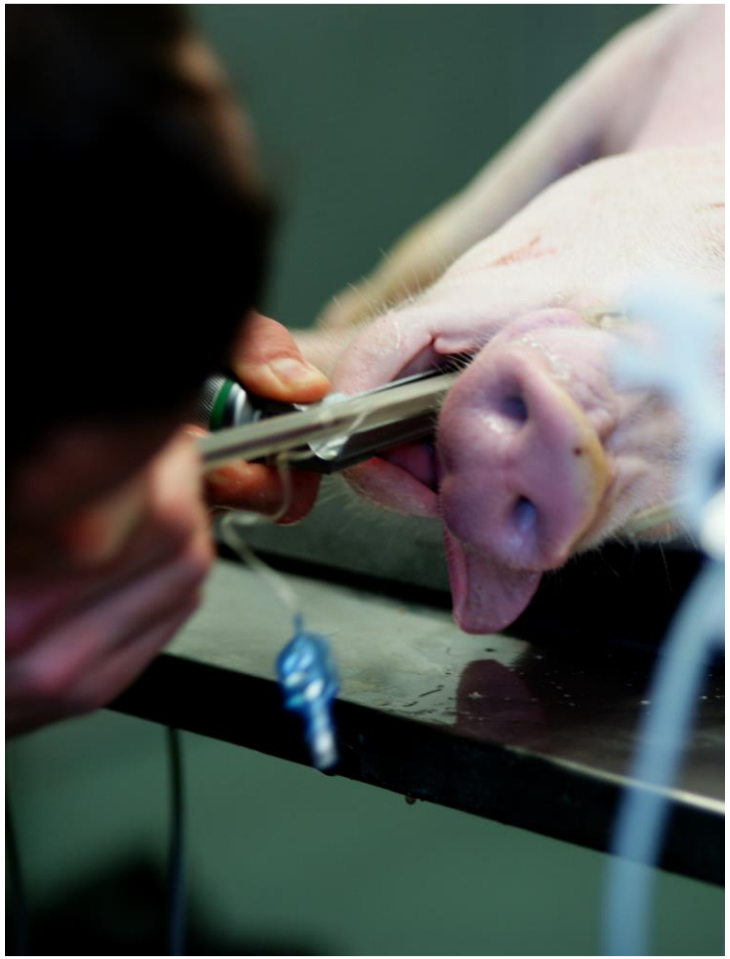
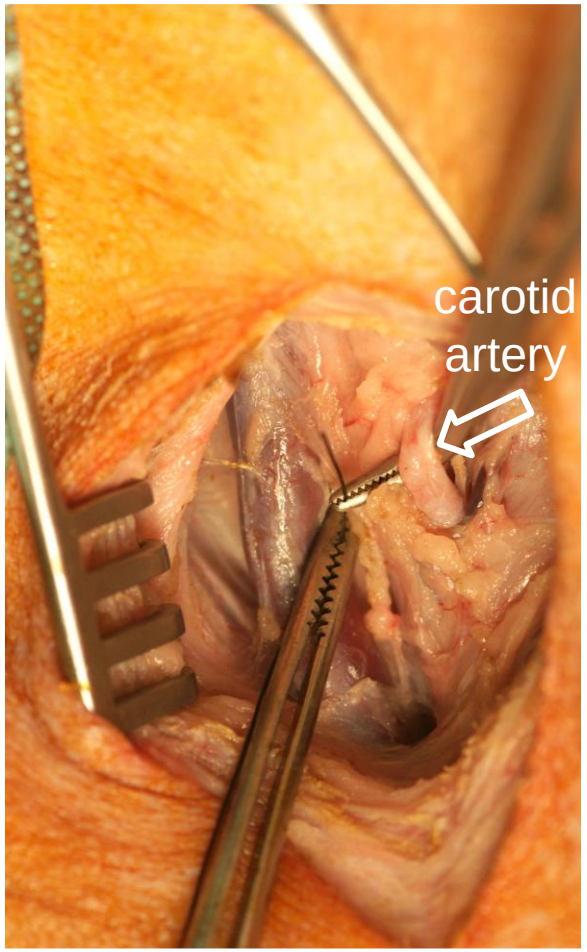
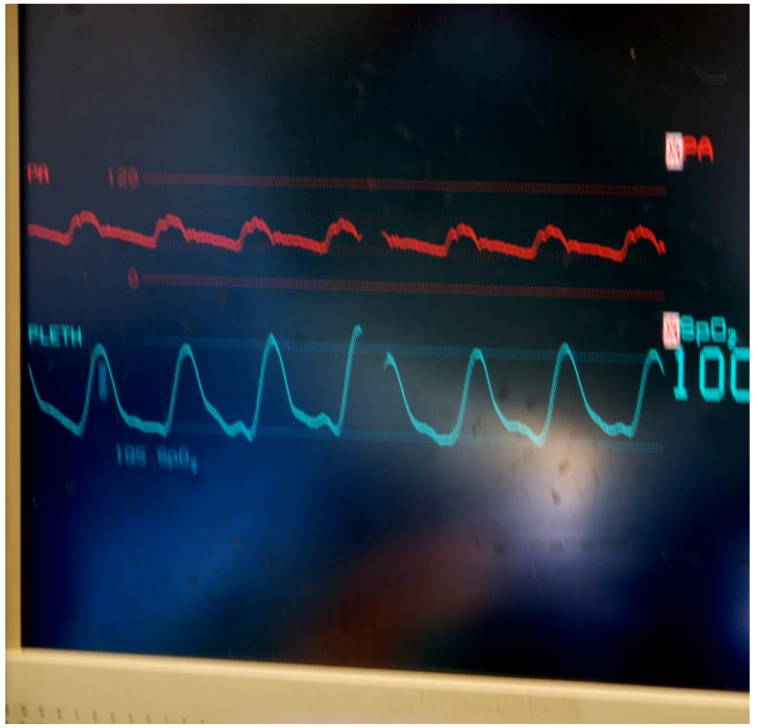
Recipient preparation



Oro-tracheal intubation



Arterial pressure monitoring



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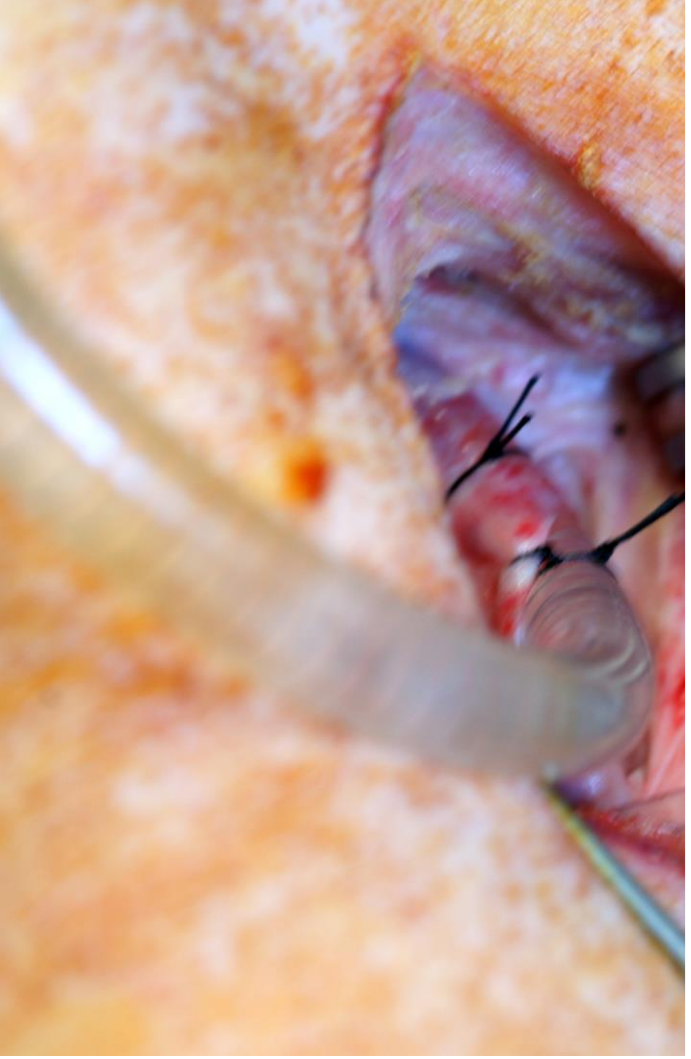
Shunt placement prior to liver removal



Jugular vein cannulation

Connection to the shunt tubing

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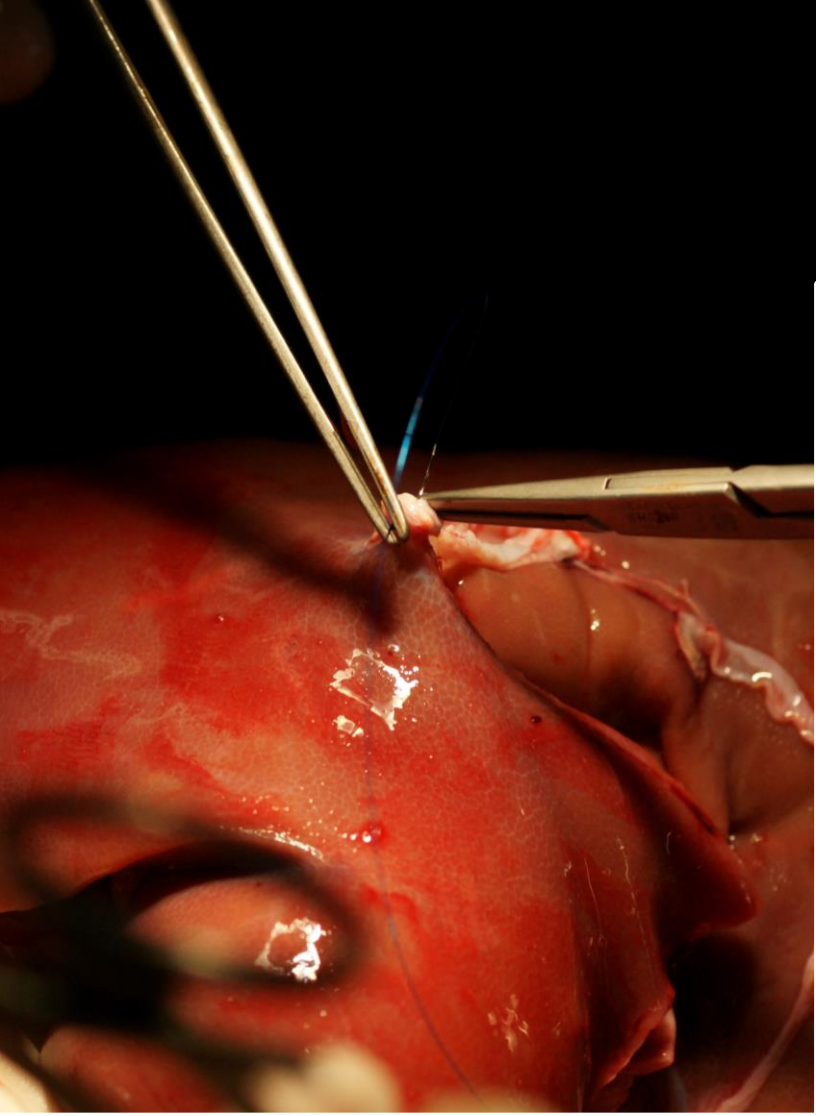
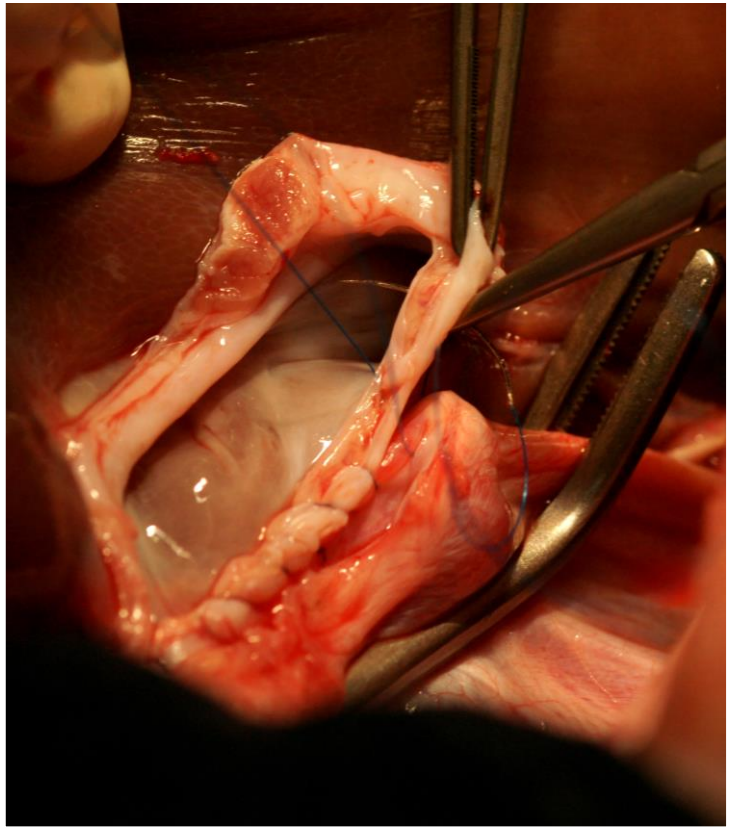
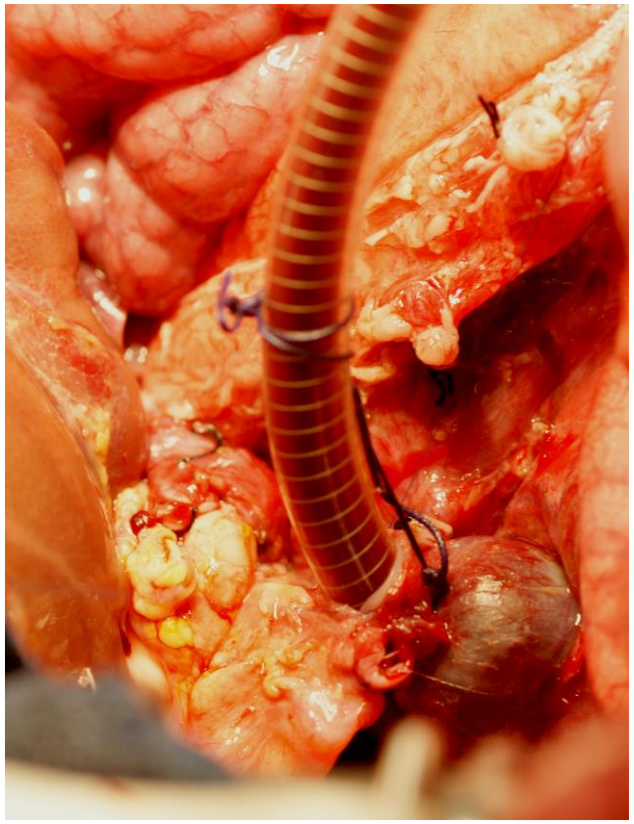
Liver implantation





Venous implantation takes place during the anhepatic phase



Portal anastomosis

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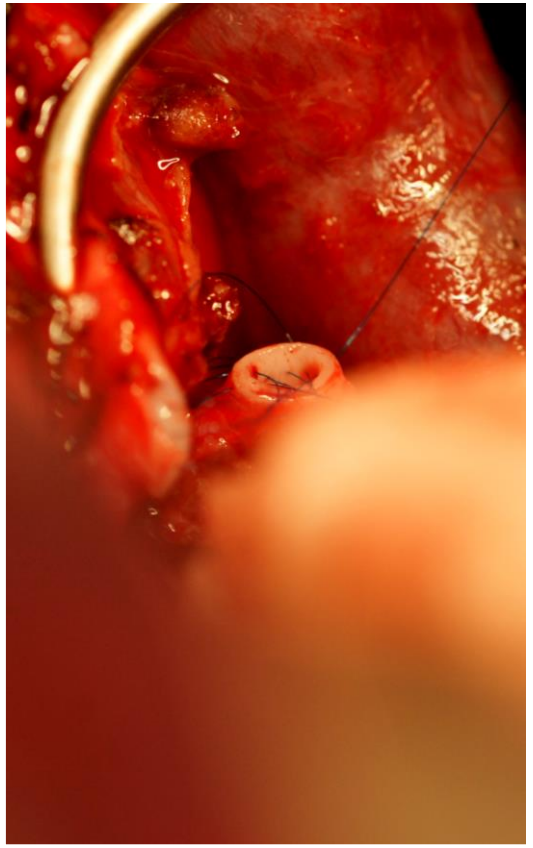
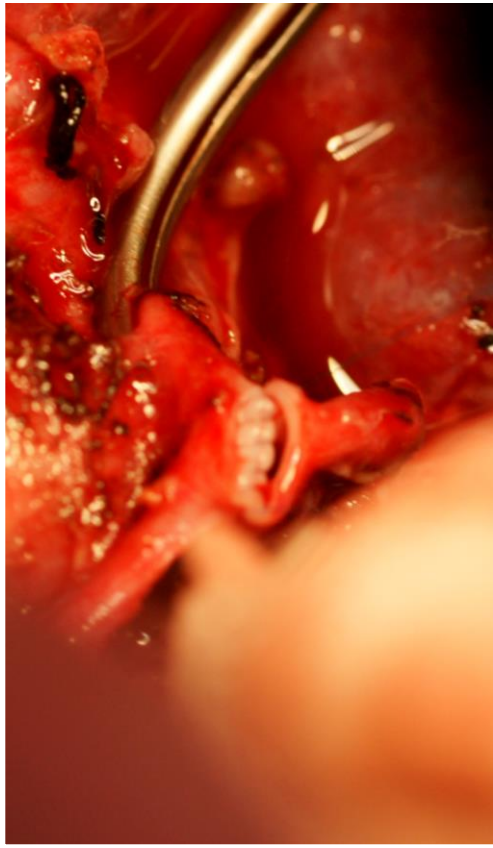
Reperfusion

The shunt is removed and blood flow allowed back into the liver





Arterial anastomosis

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Surgical procedure report

- Operative durations, cold ischemia and fluid infusion were similar
- Arterial pressure monitoring showed similar variations in all recipients
- No difficulty during surgeries and all recipients survived the procedure

Key figures:	recipient operation:	3 hours
	cold ischemia:	3 hours
	anhepatic phase:	40 minutes

Detailed information in paper report

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Results

- At this time there is no claim on the effect of nanoparticles
- No statistical test performed
- We did not observe any adverse reaction to nanoparticle treatment

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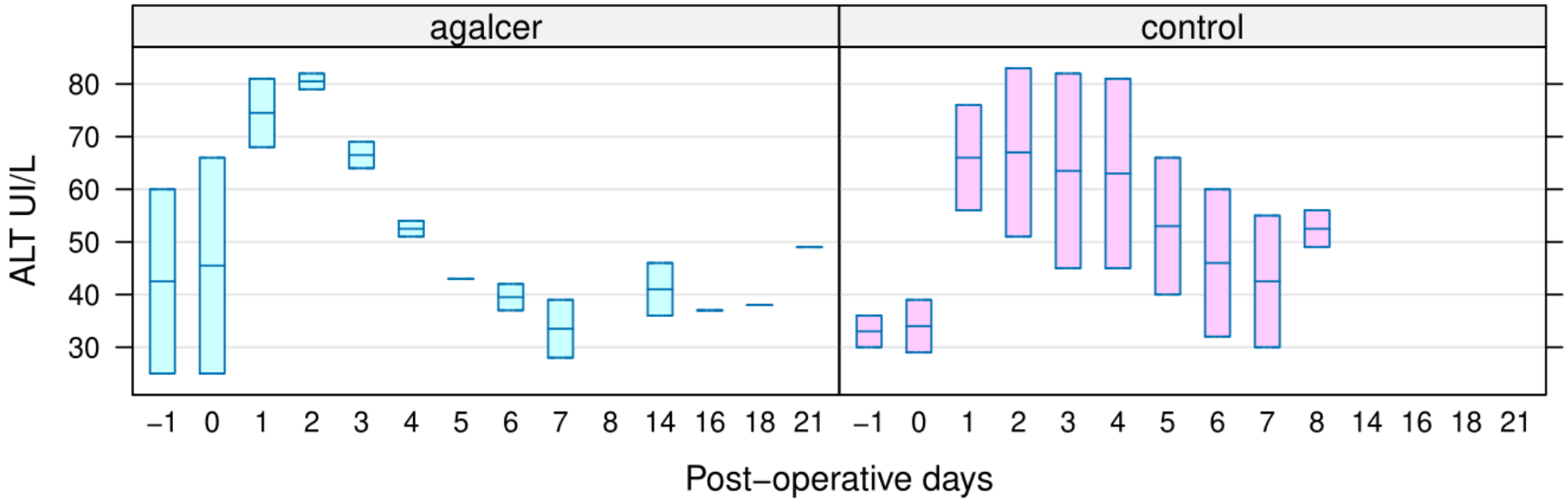
Results: biochemical data

- n = 4
- Markers of cellular injury : AST ALT LDH Lactate
  - Rapid elevation with a maximum on day 1 or 2, slow normalisation
  - Consistent with reperfusion injury



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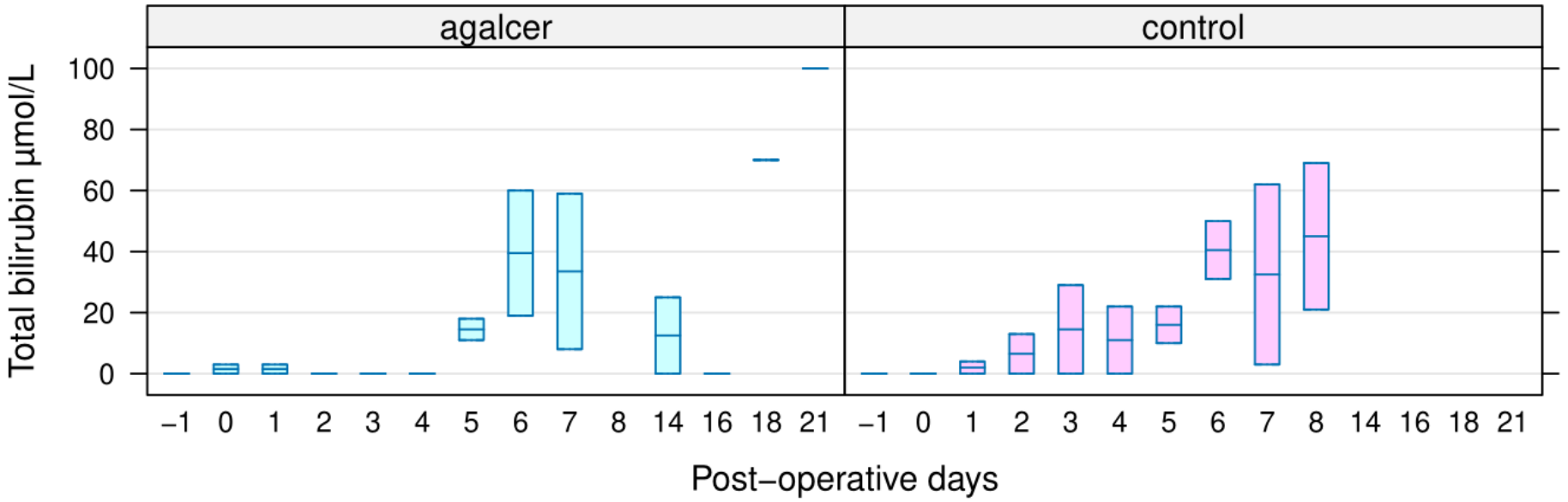
Results: biochemical data

- Markers of cholestasis: GGT, Alkaline Phosphatase, Total bilirubin
  - Slow elevation towards the end of the first week



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Results: biochemical data

- Kidney function: within normal values
- Albumin levels: low normal values maintained throughout follow-up
- Glucose levels: spike on post-operative sample, normal throughout follow-up

- Left to process: samples from experiments 5, 6, 7, antithrombin

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Results: biochemical data

- We observed early biochemical variations consistent with liver ischemia/reperfusion
- Slow elevation of cholestasis markers at the end of the first week is a marker of graft dysfunction with multiple possible causes, including:
  - ischemia/reperfusion injury
  - sepsis
  - technical complication (artery thrombosis, bile duct leak)
  - rejection
- The was no kidney function impairment and no difference between groups (in a sample of 4 observations)

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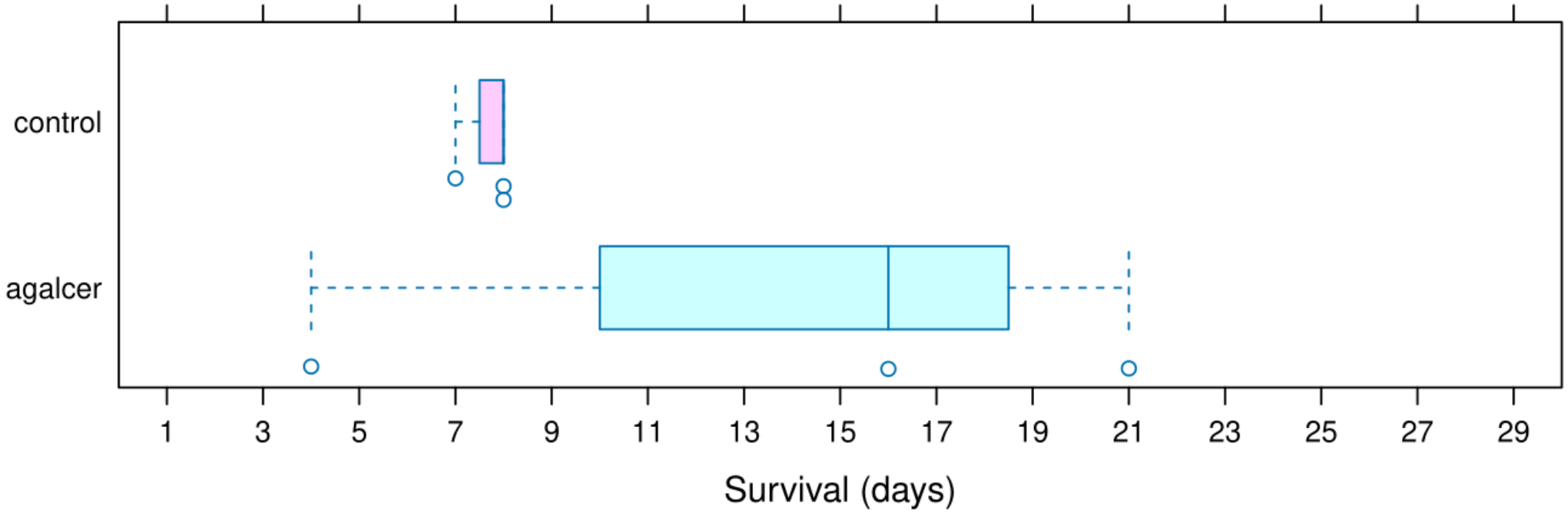
Results: recipient survival

- Experiments 1, 2, 3, 4, 5 were euthanised after reaching a humane endpoint
- Experiment 6 died unexpectedly during the night at day 3
- Median survival was 16 (agalcer) and 8 days (control)



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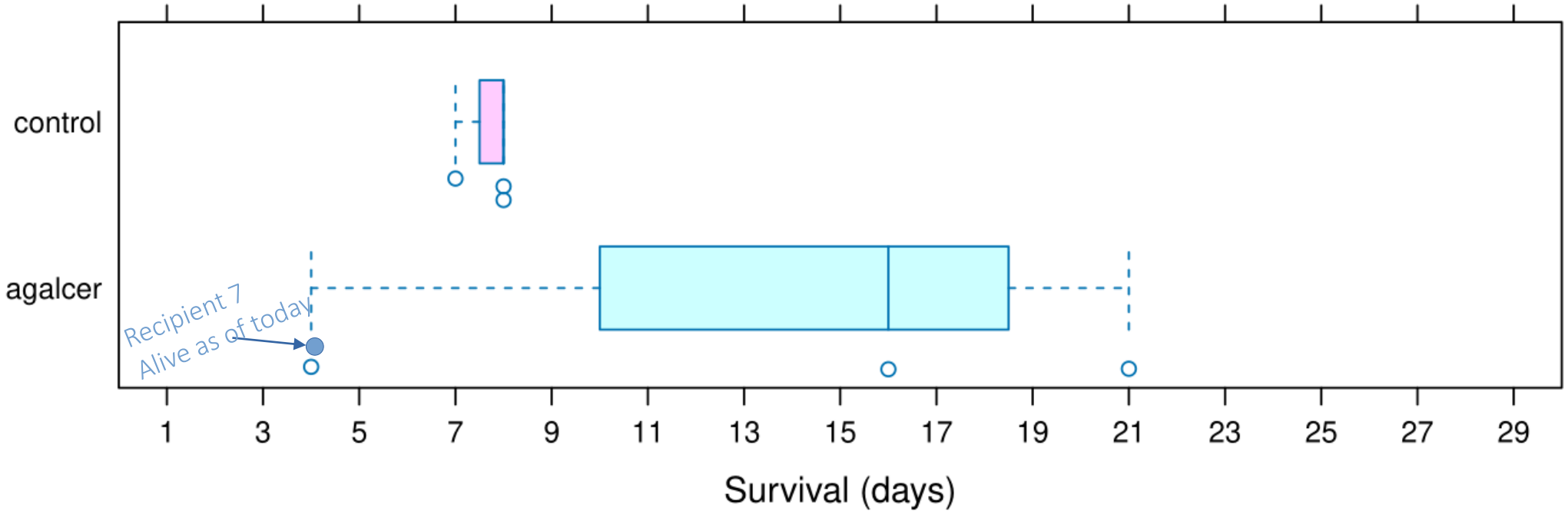

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Results: follow-up

- In the treated group 1/3 of animals received the full 10 doses
- 2 control animals exhibited fever > 40°C on days 2 and 3 which was resolutive
- Recipient 1 had a gastric ulcer which led to gastric bleeding
  - attributed to the inefficacy of the oral route for PPIs
- All recipients exhibited during the end of follow-up a state of listlessness associated with anorexia triggering euthanasia

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Results: necropsies

- The majority of animals had ascites
- 2 animals had a surgical complication (bile duct leak)
  - Recipient 5 also had bile peritonitis
  - Recipient 6 had no ascites or peritonitis

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Results: biopsies

- Two scores were used to grade lesions
- Suzuki score: liver injury, 3 parameters
  - Sinusoidal congestion
  - Cytoplasm vacuolisation
  - Parenchymal necrosis
- Banff Rejection Activity Index: 3 parameters
  - Portal inflammation
  - Venous endothelial inflammation
  - Bile duct damage

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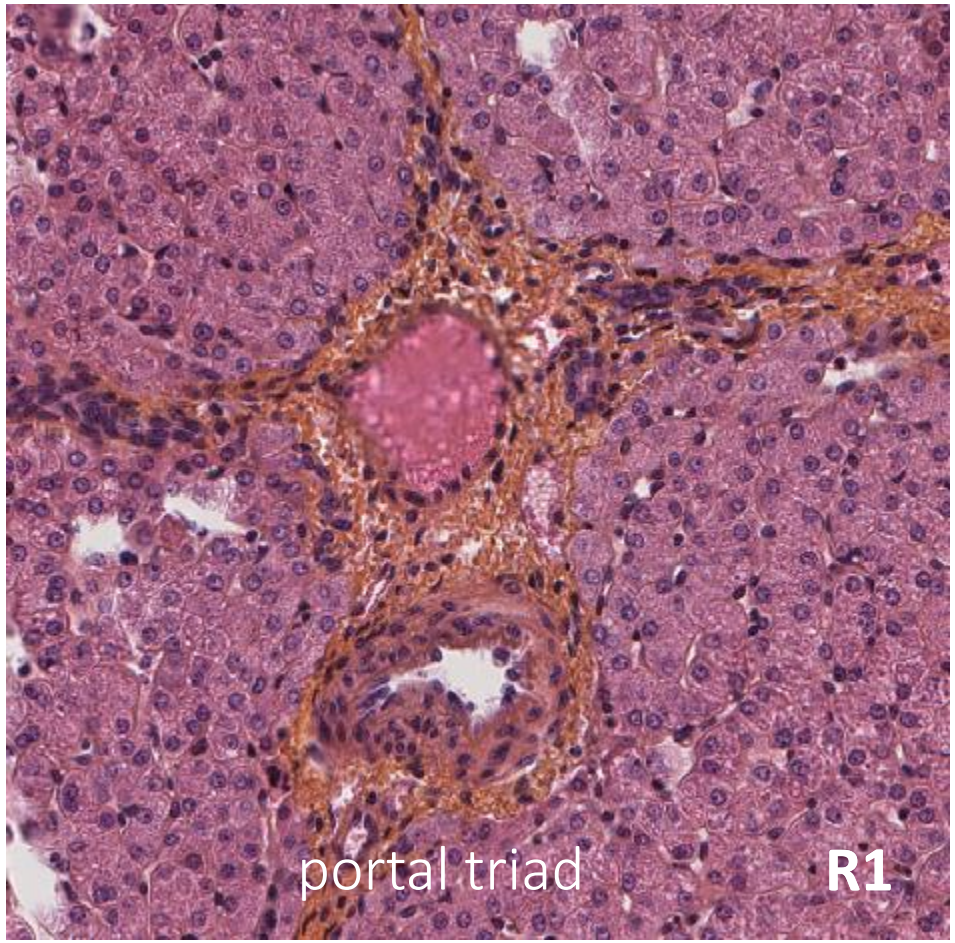
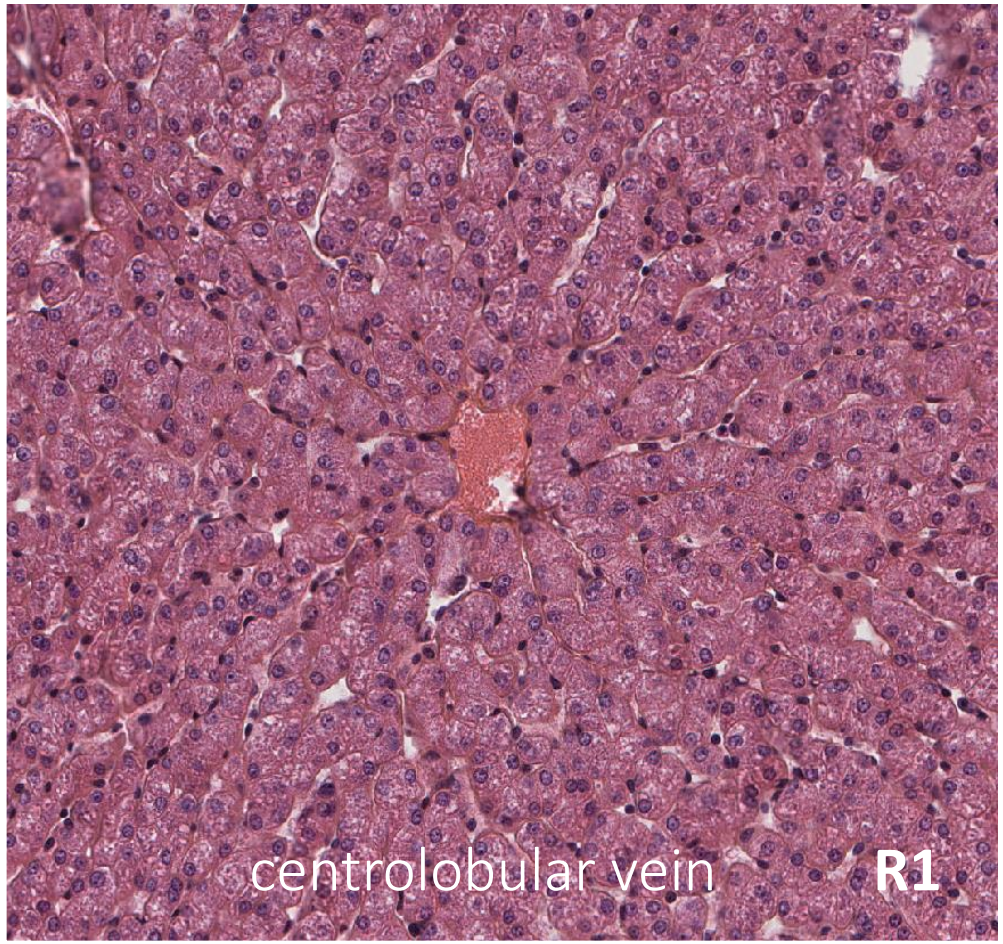
Results: biopsies

- Post-reperfusion biopsies show no anomalies





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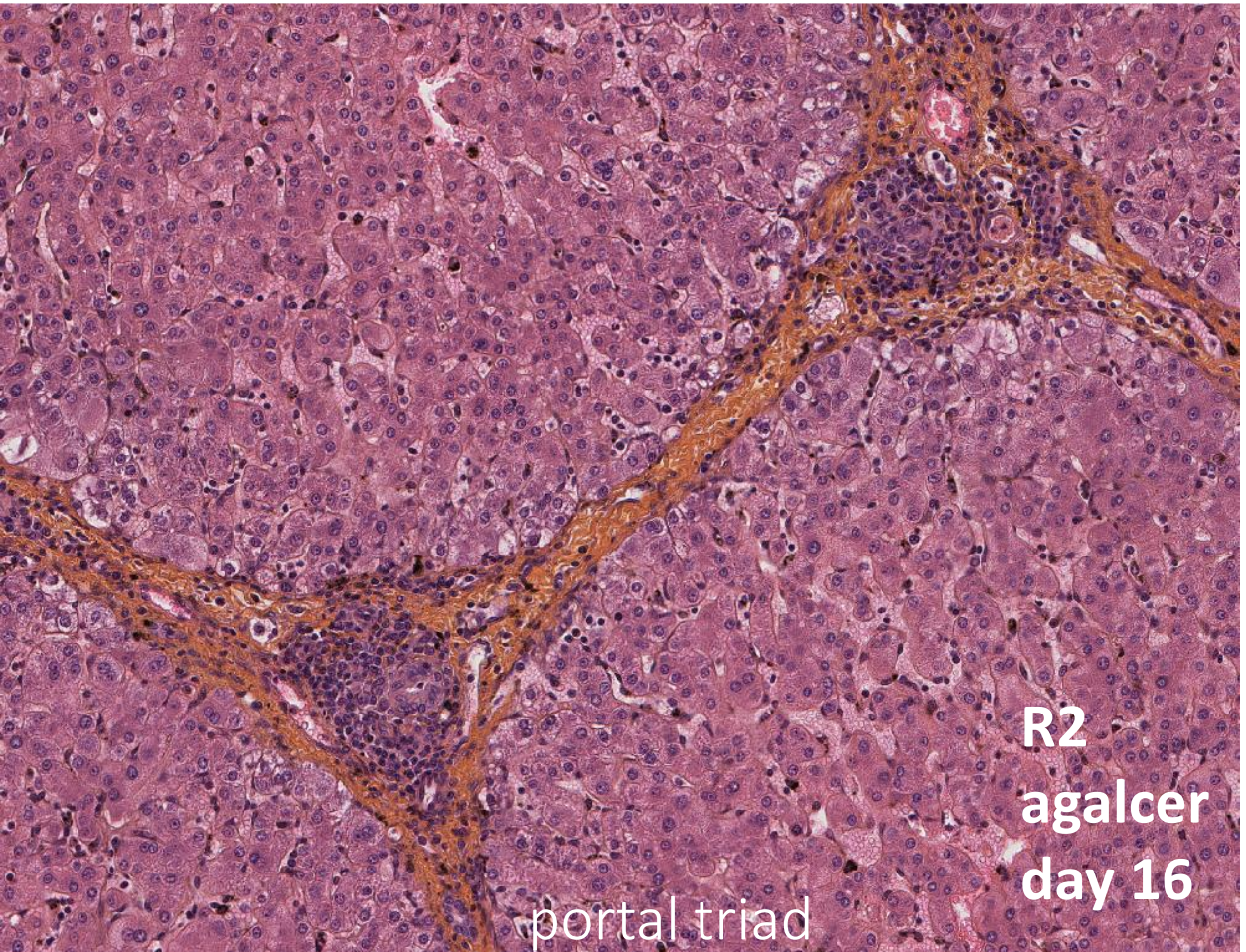
Results: biopsies

- No evidence of acute T-cell mediated rejection in any post-mortem biopsy
  - Mild portal inflammation indicative of alloreaction
  - No venous endothelial inflammation
  - No bile duct damage



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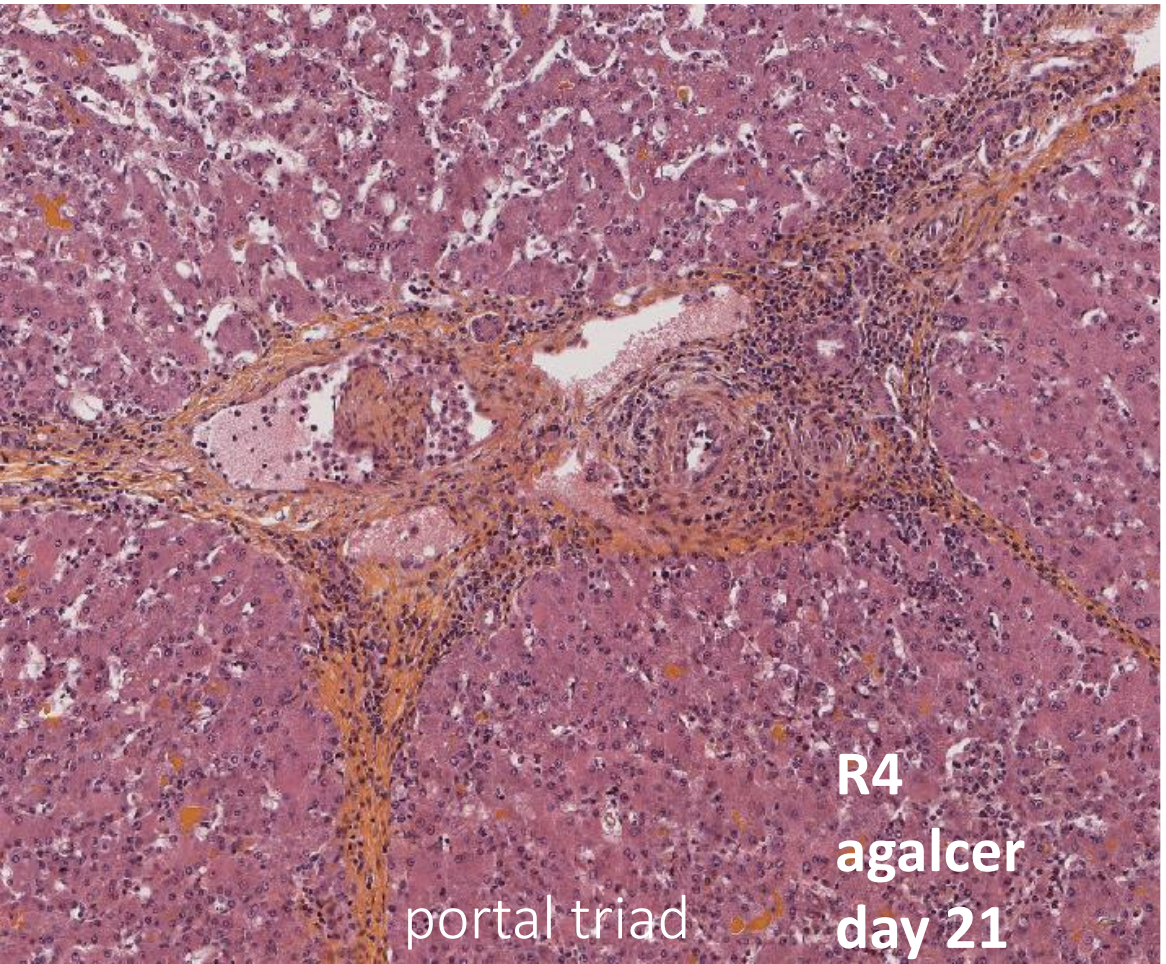
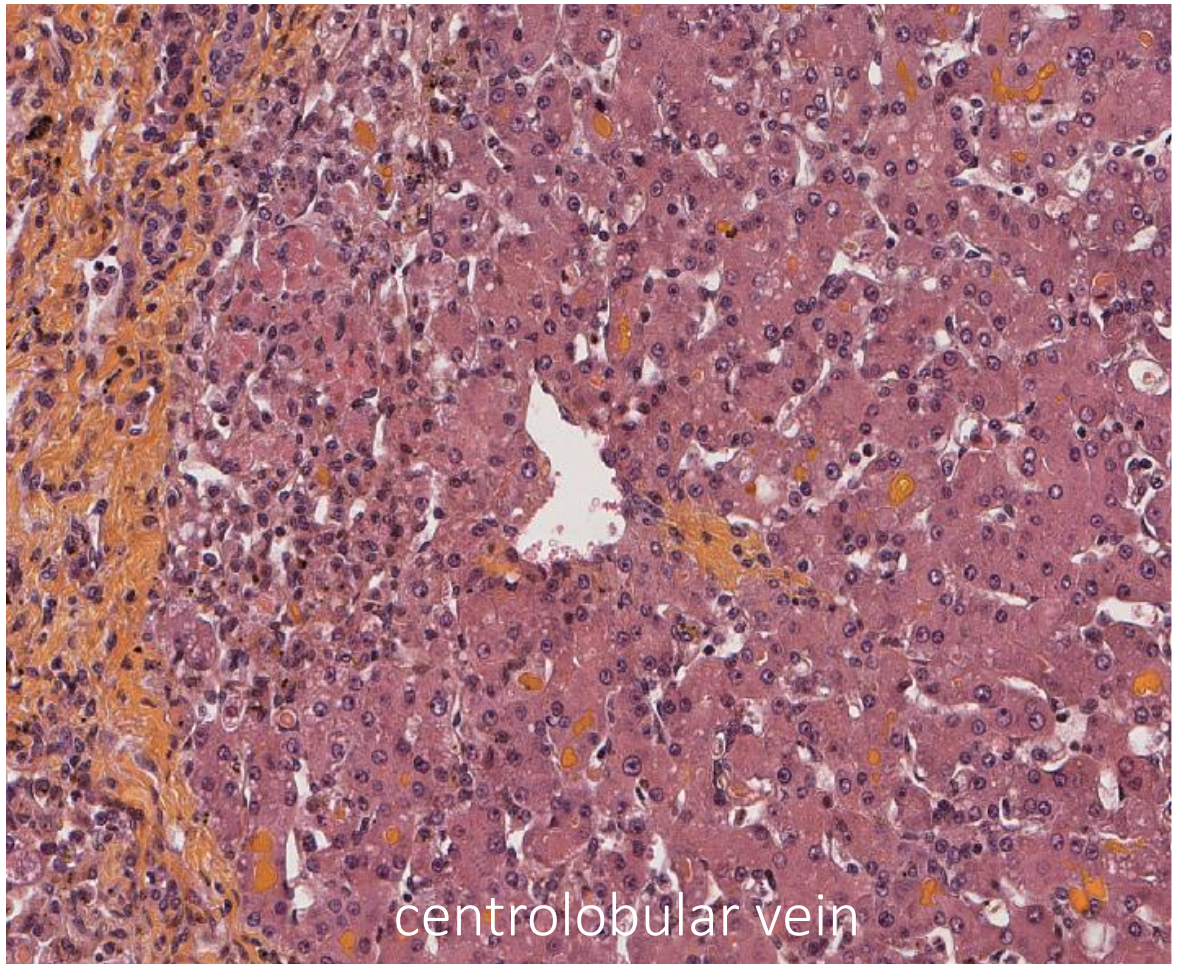
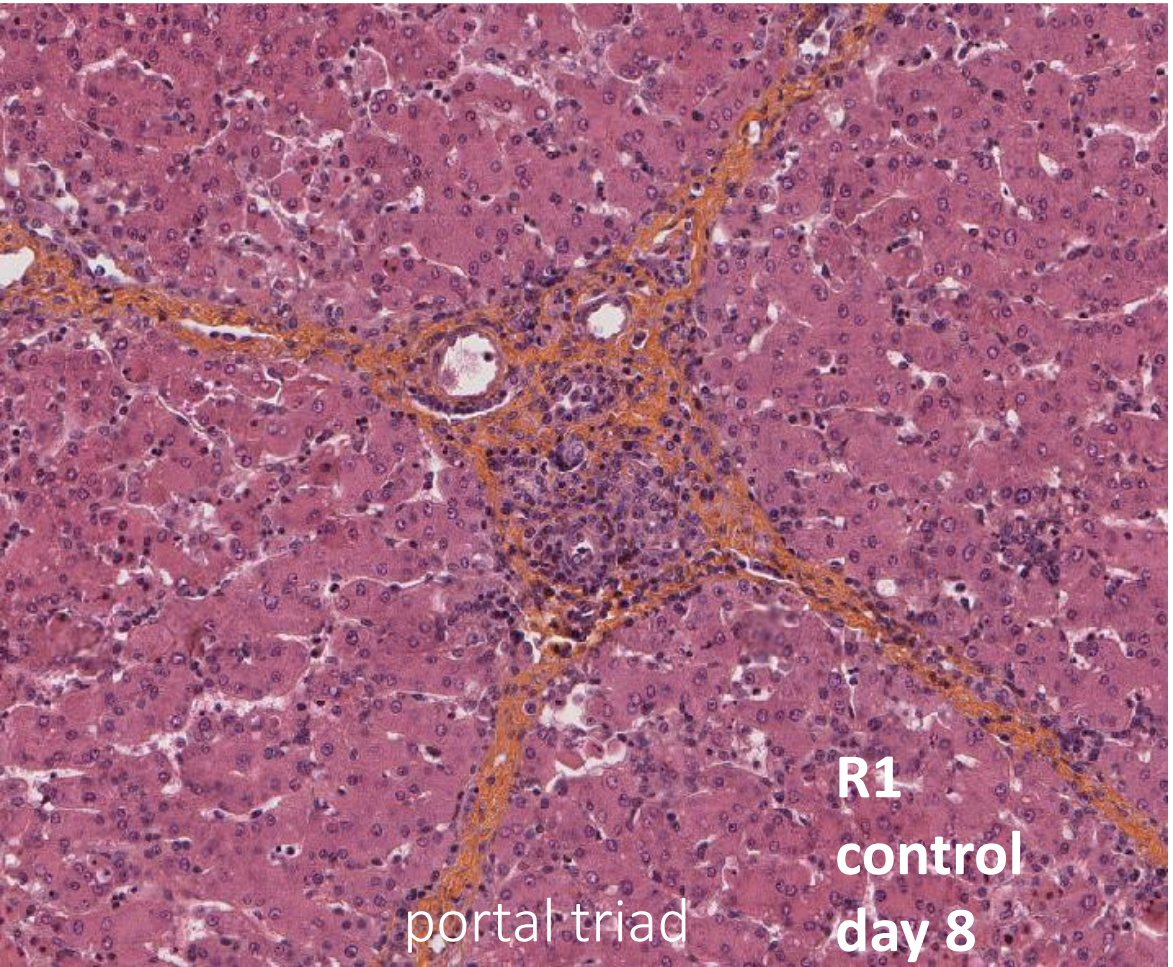
Results: biopsies

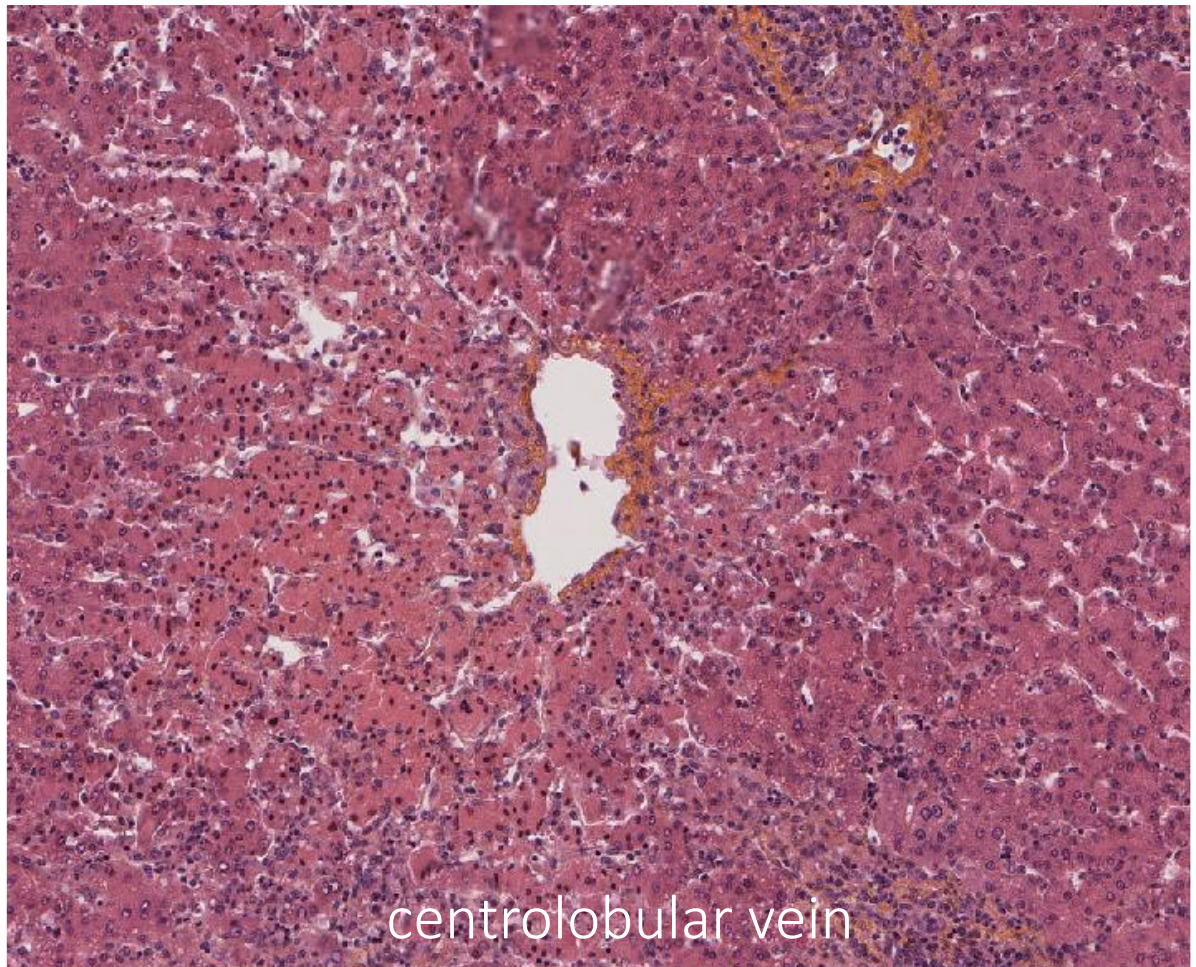




Results: biopsies

- Banff Rejection Activity Index: similar findings in the control group





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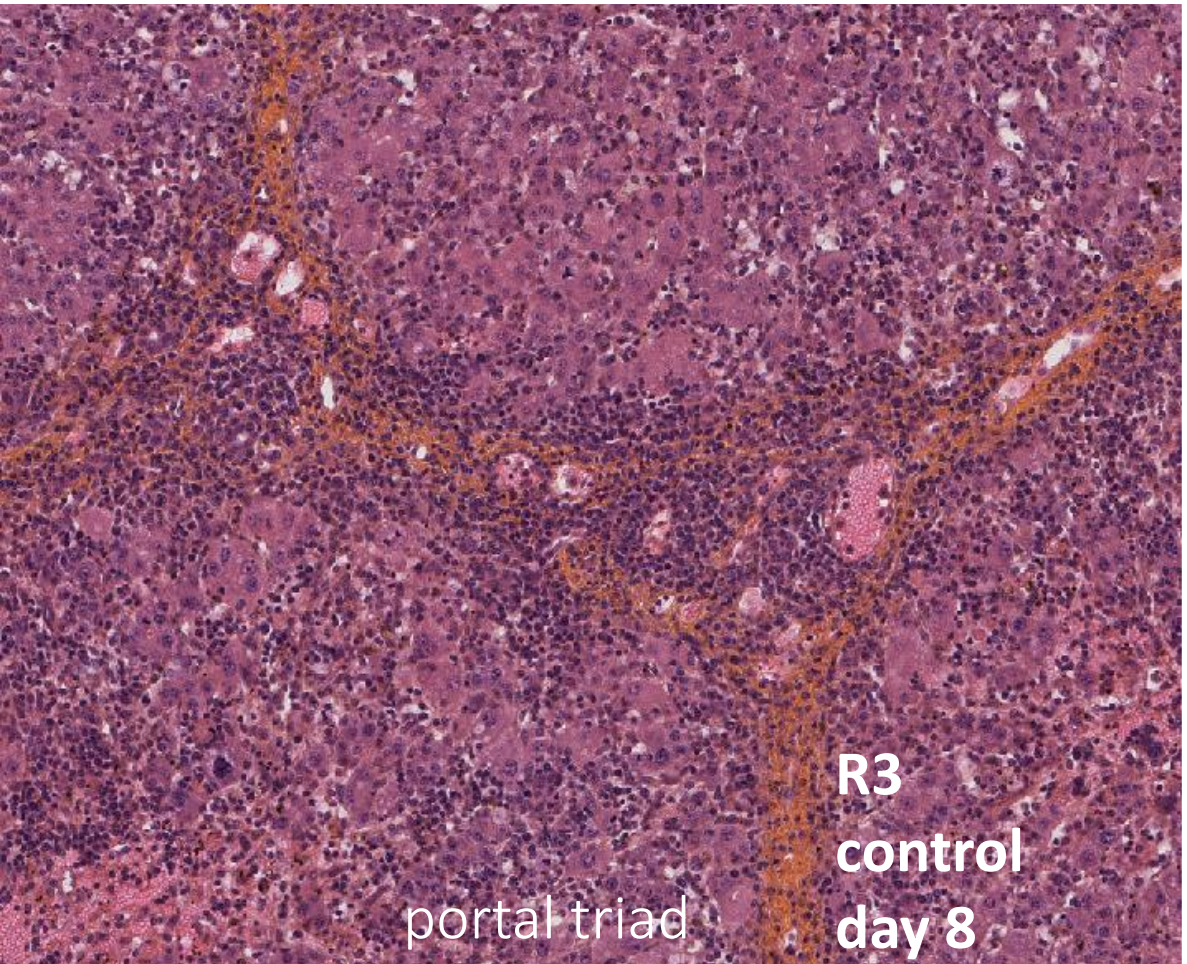
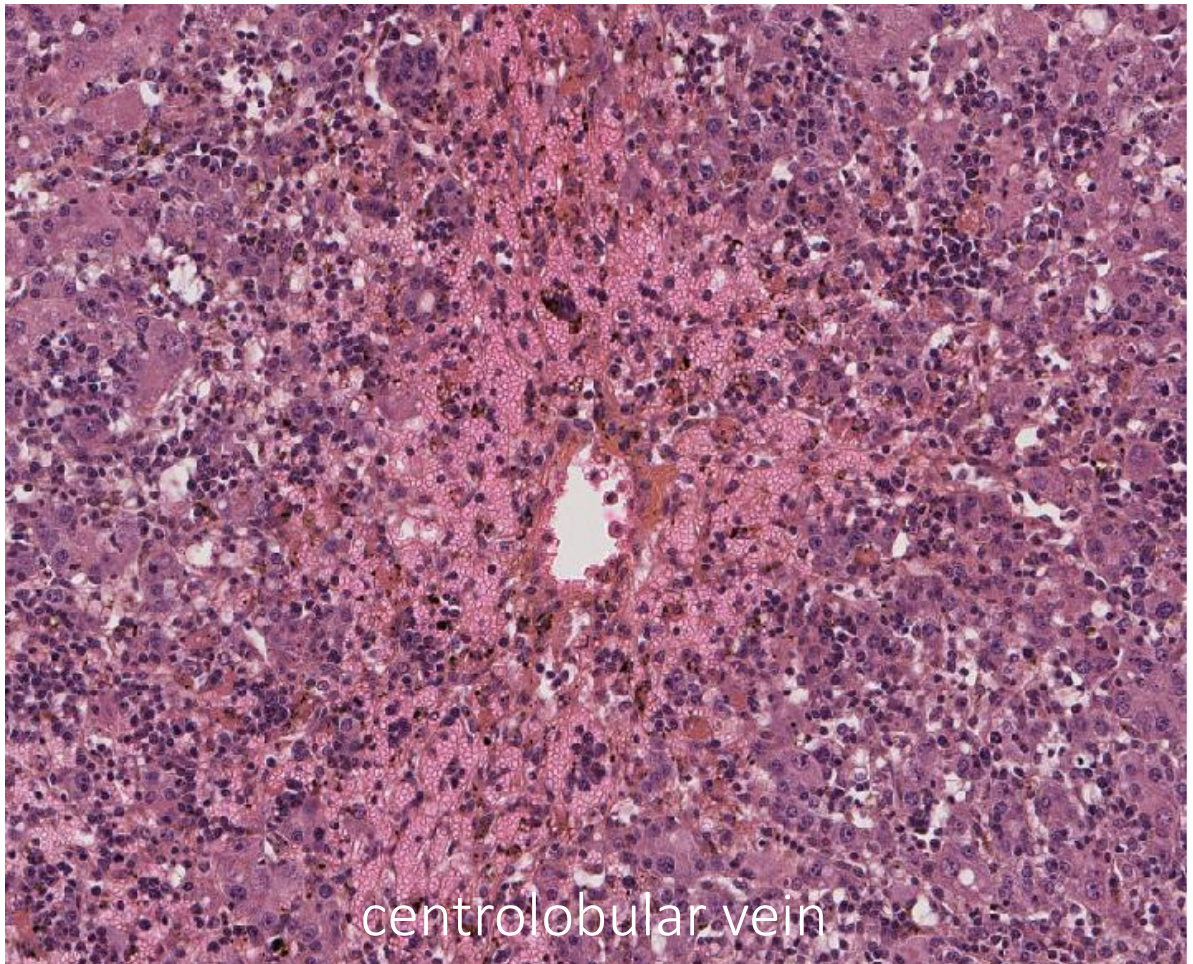
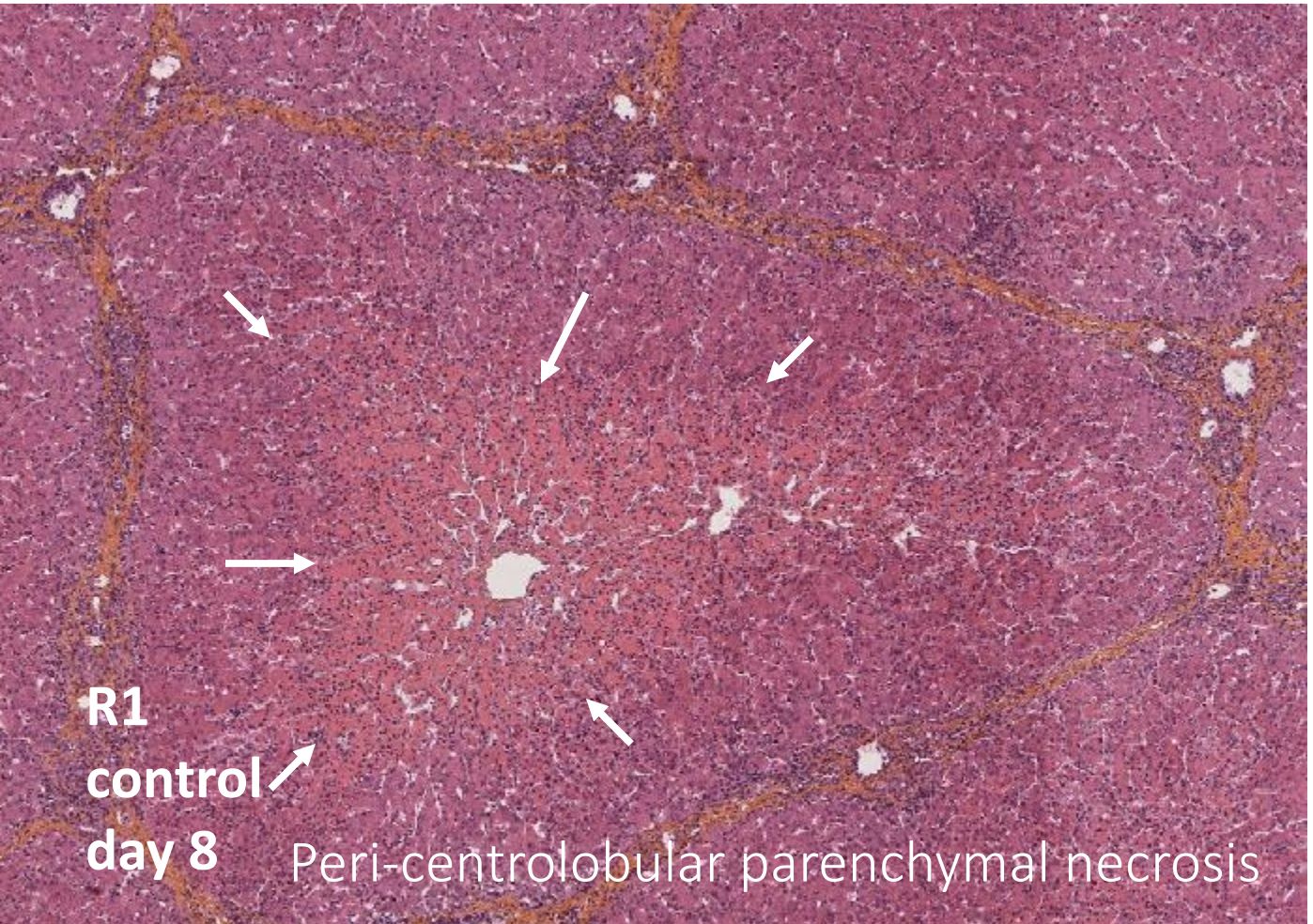
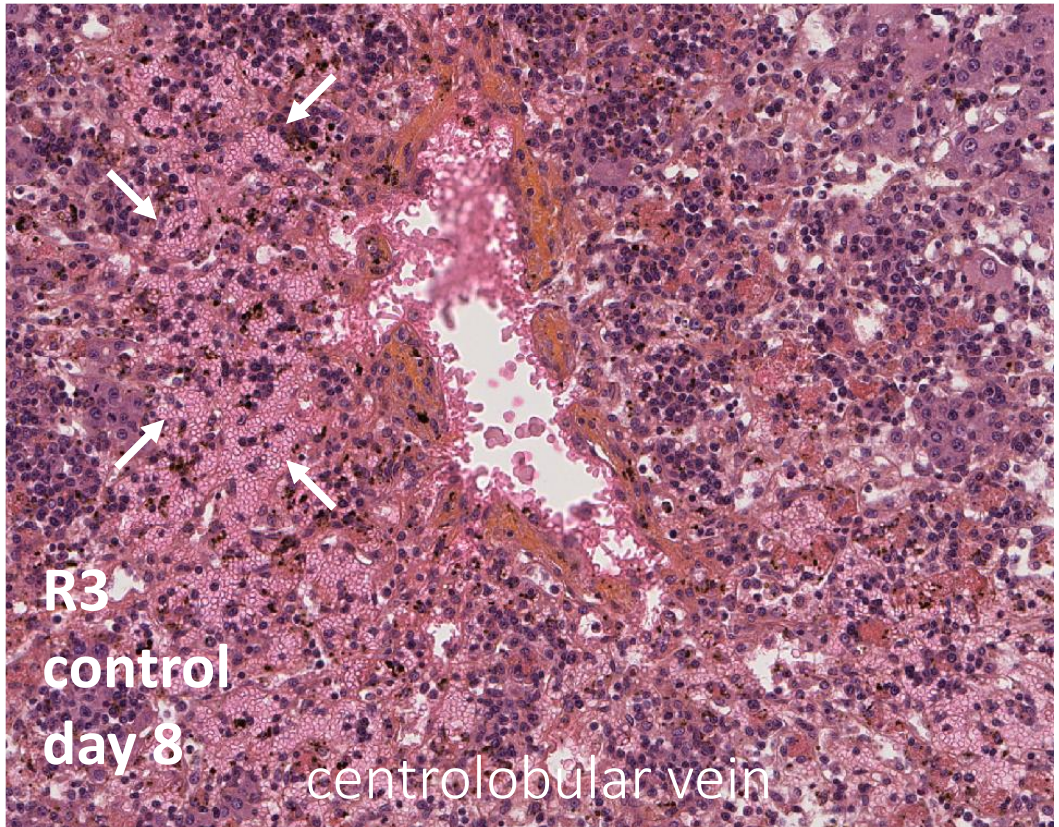




Results: biopsies

- Suzuki score: marked injury in some samples
  - Sinusoidal congestion
  - Parenchymal necrosis





Results: biopsies

- Banff score for all post-mortem biopsies: 1/9 (indeterminate)
- Suzuki score: all recipients except no. 2 (agalcer) showed significant injury (4-5/12)

		Suzuki score				Banff Rejection Activity Index			
id	Survival (days)	SC	CV	PN	Total	PI	VEI	BDD	RAI
agalcer									
02	16	None	None	None	0	1	0	0	Indeterminate
04	21	Mild	None	< 60 %	5	1	0	0	Indeterminate
control									
01	8	None	None	$> 60\%$	4	1	0	0	Indeterminate
03	8	Mild	None	$< 30\%$	4	1	0	0	Indeterminate

Abbreviations:
BDD: bile duct damage; CV: cytoplasm vacuolisation; PI: portal inflammation; PN: parenchymal necrosis; SC: sinusoidal congestion; RAI: rejection activity index; VEI: venous endothelial inflammation.

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Careful interpretation of results

- There was longer survival in the treated group
- No nanoparticle toxicity was observed
- No rejection was observed in any of the samples
- Moderate graft injury was observed in both groups which can be explained by one or more of the following causes:
  - ischemia/reperfusion injury
  - surgical complication
  - infection

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T3.2: iNKT isolation

- Second dry run + isolation of lymphocytes from recipient 5
- Samples were shipped to IDIBAPS for analysis

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Liver iNKT isolation

Results obtained with pig's samples

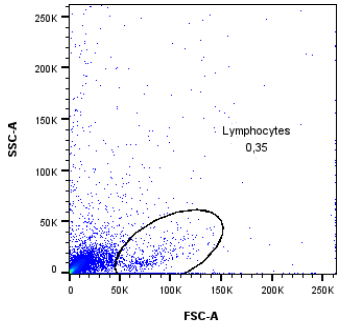
C

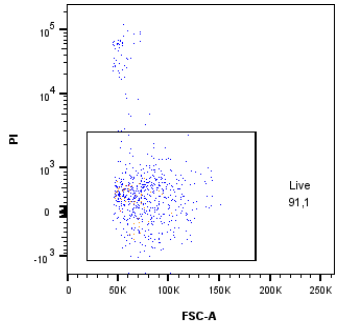
Liver flush → Liver disruption → Percoll gradient → No CD3 enrichment → Staining

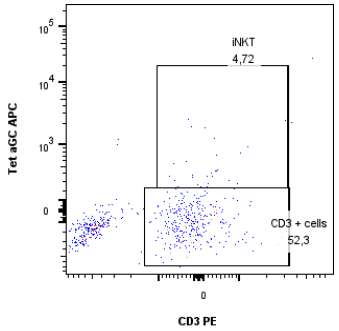
Samples from pig's livers frozen and sent by Alexander. In the Neubauer chamber's counts we have seen just 160.000 cells, in a tube that Alexander says that there were 4.4000.000 cells.

Note: **Alexander said that has flushed the samples with PBS-EDTA 5x. Maybe he has damaged the cells**

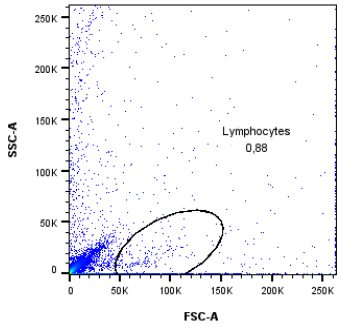
With PI

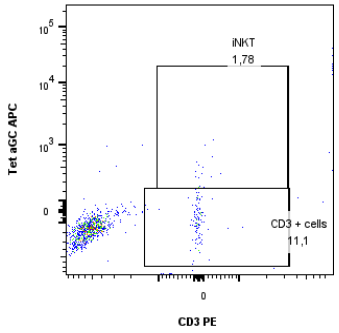


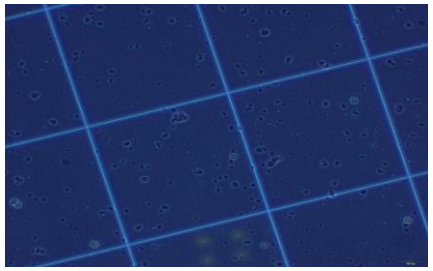




Without PI

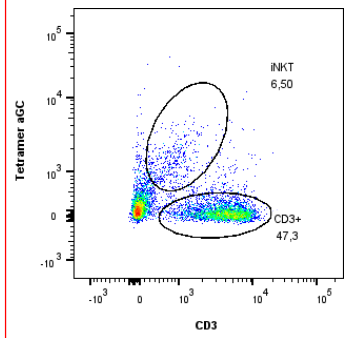


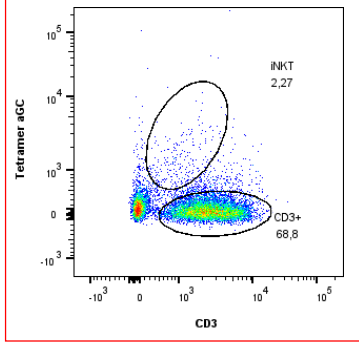




Neubauer's chamber

Frozen cells from mouse



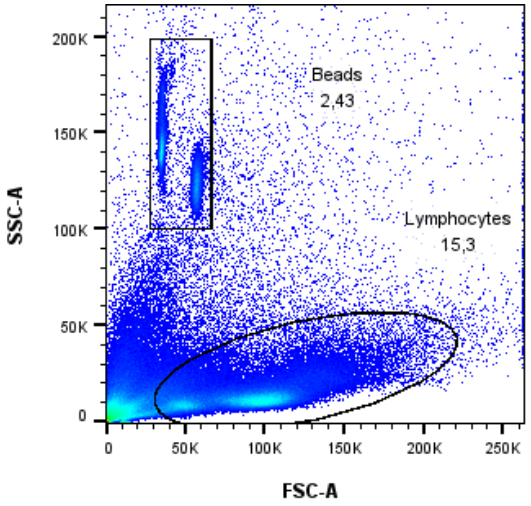


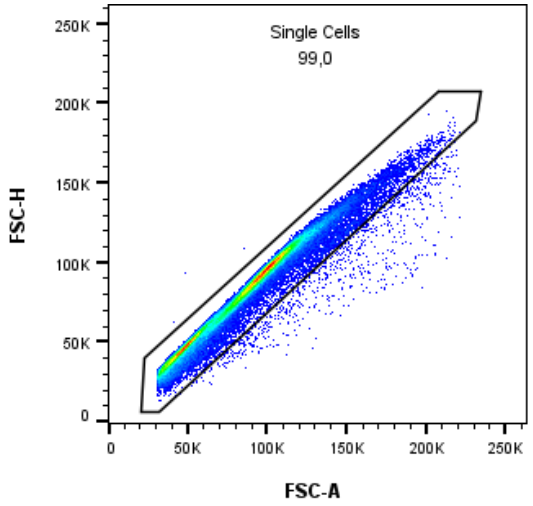
Liver iNKT isolation

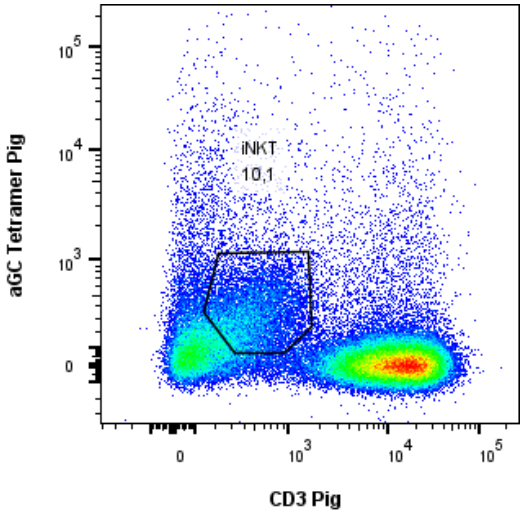
Dry run sample 11/06/2025

C

Liver flush → Liver disruption → Percoll gradient → Freeze and sent → CD3 enrichment → Staining







Forward focus

- We need to collect data from the remaining experiments if we want to draw conclusions
- Batch 2 of NPs (30 doses) will be shipped next week to be used in July
- Discussion of dosage increase for future recipients

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