NLC Adoptive Transfer (Liver-derived NK-like Cells)

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Purpose

This protocol describes adoptive transfer of **liver-derived NLCs (NK-like cells)** into recipient mice, with emphasis on:

- Expected **donor yield per liver** and planning calculations for donor requirements
- Target **NLC transfer dose** per recipient
- Standard experimental timelines for:
  - LCMV-based experiments with prior P14 transfer
  - BDL + LCMV-WE experiments

Critical notes (read before starting)

- NLC recovery varies by infection model, timepoint, operator, and digestion quality; plan donors with buffer.
- Keep cells cold after final wash and minimize time between final resuspension and injection.
- Use congenic markers (CD45.1/CD45.2) where possible to track transferred NLCs.
- Final injection buffer should be sterile PBS unless internal SOP specifies otherwise.
- All procedures must comply with approved animal licences and biosafety regulations.

Approximate timing

- Donor liver harvest to single-cell suspension: ~**2–2.5 h** (see liver digestion protocol)
- Enrichment / sort (if used): ~**30–180 min** (method-dependent)
- Counting + preparation for injection: **10–20 min**
- Transfer (tail vein i.v.): ~**1–2 min per mouse**

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Procedure

Standard yields and transfer dose (for planning)

Expected donor yield

- **Expected NLC yield per donor liver: 5,000–8,000 NLCs per mouse liver**

Target recipient dose

- **Adoptive transfer dose: 60,000–80,000 NLCs per recipient**

Donor requirement planning table

Assumptions for planning: - Low yield = **5,000 NLCs per donor liver** - High yield = **8,000 NLCs per donor liver** - Low transfer dose = **60,000 NLCs per recipient** - High transfer dose = **80,000 NLCs per recipient** - Donor livers required are rounded **up** to whole livers

Recipient transfer dose	Donor yield per liver	Donor livers needed **per recipient**	Example calculation
60,000	8,000	8	60,000 / 8,000 = 7.5 → **8**
60,000	5,000	12	60,000 / 5,000 = 12 → **12**
80,000	8,000	10	80,000 / 8,000 = 10 → **10**
80,000	5,000	16	80,000 / 5,000 = 16 → **16**

Practical note: For multi-recipient experiments, multiply the "donor livers per recipient" by the number of recipients, then add an additional buffer (recommended) to account for losses during processing and sorting.

Donor cell preparation (high-level)

1. Harvest donor livers from mice at the defined experimental timepoint.
2. Prepare single-cell suspensions using the lab-standard liver digestion workflow (perfusion, enzymatic digestion, density enrichment, RBC lysis as needed).
3. Enrich or sort NLCs according to the experimental design (bulk enrichment vs FACS for high purity).
4. Count viable cells and prepare the final injection suspension in sterile PBS.
5. Transfer **60,000–80,000 NLCs per recipient** by i.v. tail vein injection.

Intravenous adoptive transfer parameters

- **Route:** i.v. (tail vein)
- **Injection volume: 200 µL per mouse**
- **Needle:** insulin syringe, typically **27G–30G**
- Keep cells on ice and gently mix to maintain a uniform suspension during injection series.

Experiment timelines

LCMV experiment timeline (P14 → LCMV → NLC transfer)

Design: - P14 transfer on Day 0 - LCMV infection on Day 1 - NLC transfer **20 days after infection** - Assessment **5 days after NLC transfer**

Timeline table (absolute and relative)

Absolute day	Relative description	Event
Day 0	Start	Transfer P14 cells
Day 1	**24 hours later**	Infect with LCMV
Day 21	**20 days after infection**	Transfer NLCs
Day 26	**5 days after NLC transfer**	Harvest / assessment (organs, flow, etc.)

BDL + LCMV-WE infection timeline (BDL → LCMV → NLC transfer → harvest)

Design: - BDL on Day 0 - Infect with LCMV-WE approximately 9 days later - Transfer NLCs at **4–6 days post infection (dpi)** - Harvest organs at **9 dpi**

Timeline table (absolute and relative)

Absolute day	Relative description	Event
Day 0	Start	BDL surgery
Day 9	**~9 days later**	Infect with LCMV-WE
Day 13–15	**4–6 dpi**	Transfer NLCs
Day 18	**9 dpi**	Harvest organs for analysis

Interpretation of spacing: - If NLC transfer occurs at **4 dpi**, harvest at 9 dpi is **5 days after transfer**. - If NLC transfer occurs at **6 dpi**, harvest at 9 dpi is **3 days after transfer**. - Choose dpi for transfer based on the desired window between transfer and analysis.

Typical readouts (minimal set)

- Tracking of transferred NLCs by congenic markers (frequency, phenotype) in liver ± spleen/blood
- Recipient immune context (key lymphoid/myeloid compartments as relevant to model)
- Functional assays as needed (e.g., ex vivo stimulation / cytokines, cytotoxicity surrogates)
- Infection-context readouts (use lab-standard viral load or surrogate readouts where applicable)

Safety (brief)

- All animal procedures must comply with approved animal licences and institutional regulations.
- Wear appropriate PPE (lab coat, gloves, eye protection) when handling animals, tissues, and reagents.
- Handle sharps (needles, scalpels) with care and dispose of immediately in approved sharps containers.
- Perform tissue and cell handling in a biological safety cabinet where appropriate.
- Dispose of viral waste and contaminated materials according to local biosafety regulations.

Version history

Version	Date	Author	Change summary
v1.0	2026-01-09	Dillon Corvino	Initial NLC adoptive transfer protocol (planning yields, donor requirements, and standard timelines for LCMV and BDL+LCMV designs)