
Book Section:
Brownlie, R.J., Zamoyska, R. and Salmond, R.J. orcid.org/0000-0002-1807-1056 (2024) OT-I TCR Transgenic Mice to Study the Role of PTPN22 in Anti-cancer Immunity. In: Protein Tyrosine Phosphatases Methods and Protocols. Methods in Molecular Biology, 2743 . Springer US , New York, NY , pp. 81-92. ISBN 978-1-0716-3568-1

https://doi.org/10.1007/978-1-0716-3569-8_5

OT-I TCR transgenic mice to study the role of PTPN22 in anti-cancer immunity

Running head – Mouse models for the study of PTPN22 function in T cells

Rebecca J. Brownlie[1], Rose Zamoyska[2], Robert J. Salmond[1]

1. Leeds Institute of Medical Research at St James's, University of Leeds, Wellcome Trust Brenner Building, St James's University Hospital, Leeds, UK.
2. Institute of Immunology and Infection Research, University of Edinburgh, Ashworth Laboratories, Edinburgh, UK
Correspondence to: Robert J. Salmond, E-mail: r.j.salmond@leeds.ac.uk

i) Chapter Title: OT-I TCR transgenic mice to study the role of PTPN22 in anti-cancer immunity

ii) Abstract

Phosphotyrosine phosphatase non-receptor type 22 (PTPN22) is a key regulator of immune cell activation and responses. Genetic polymorphisms of *PTPN22* have been strongly linked with increased risk of developing autoimmune diseases whilst analysis of PTPN22-deficient mouse strains has determined that PTPN22 serves as a negative regulator of T cell antigen receptor signalling. As well as these key roles in the maintenance of immune tolerance, PTPN22 acts as an intracellular checkpoint for T cell responses to cancer, suggesting that PTPN22 might represent a useful target to improve T cell immunotherapies. To assess the potential for targeting PTPN22, we have crossed *Ptpn22*-deficient mice to an OT-I TCR transgenic background and used adoptive T cell transfer approaches in mouse models of cancer. We provide basic methods for the *in vitro* expansion of effector OT-I cytotoxic T lymphocytes, *in vitro* phenotypic analysis and *in vivo* adoptive T cell transfer models for the assessment of the role of PTPN22 in anti-cancer immunity.

iii) Key words: PTPN22; T cell receptor; OT-I; adoptive cell transfer; immunotherapy; ovarian carcinoma

1 - Introduction

PTPN22 is a cytoplasmic tyrosine phosphatase predominantly expressed in cells of haematopoietic origin. Interest in the cellular functions of PTPN22 has been driven by studies showing that single-nucleotide polymorphisms in *PTPN22* are significant risk factors for the development of autoimmune diseases such as rheumatoid arthritis and Type 1 diabetes (reviewed in [1-3]). Consistent with a central role for PTPN22 in immunity, over the past two decades key functions for PTPN22 in the regulation of both adaptive and innate immune cell function have been defined. Studies using *Ptpn22*-deficient mouse strains have determined that PTPN22 is a negative regulator of T cell antigen receptor (TCR) signalling [4, 5] and, in particular, plays a key role in limiting responses to low-affinity self-antigens [6]. Whilst this inhibitory function is likely important in preventing inappropriate T cell activation to self-antigens, we and others have recently shown that PTPN22 is also a negative regulator of protective T cell responses to cancer [7-11]. These data suggest that pharmacological inhibition or deletion of PTPN22 might represent a useful approach to enhancing the efficacy of cancer immunotherapies.

$CD8^+$ T cells are thought to be the major target of immunotherapies such as immune checkpoint inhibitors and play a key role in the control of malignancy by direct target cell killing and through the production of inflammatory cytokines. Our studies have determined that, in the absence of PTPN22 expression, these $CD8^+$ T cell effector functions are enhanced. To model adoptive $CD8^+$ T cell cancer therapies, we have taken advantage of the OT-I TCR transgenic mouse strain and syngeneic mouse cancer lines [7, 8]. OT-I $CD8^+$ T cells express an H-$2K^b$-restricted TCR specific for a peptide derived from chicken ovalbumin ($OVA_{257-264}$ / SIINFEKL) [12]. A useful feature of this model lies in the capacity to assess responses of the transgenic T cells to a range of well-defined variants of the SIINFEKL peptide that differ in affinity for the OT-I TCR [13]. When the OT-I strain is further crossed with knock-out, knock-in or transgenic mouse strains, this enables researchers to test the impact of genetic modifications (e.g. PTPN22-deficiency) on both high and low affinity antigen-specific $CD8^+$ T cell responses. This is particularly useful in modelling responses to tumor antigens which may vary from high affinity, immunogenic neoantigens to weakly stimulatory, low affinity self-antigens.

Here we describe basic protocols for *in vitro* expansion and differentiation of control and *Ptpn22*$^{-/-}$ OT-I effector cytotoxic T lymphocytes (CTLs). Furthermore, we describe approaches to assess CTL effector function *in vitro* and adoptive cell transfer approaches to assess anti-cancer functions of expanded T cell populations using the syngeneic ID8-OVA model of ovarian carcinoma. The protocols described here are applicable not only to the study of PTPN22 in anti-cancer immune responses, but potentially for analysis of any genetically modified mouse strain that has been crossed to the OT-I transgenic background.

2 – Materials

2.1 CTL activation and differentiation

1. Centrifuge
2. Cell culture incubator (37°C / 5% CO_2)
3. Cell counter or haemocytometer
4. 15 and 50 ml Falcon tubes
5. 70 μm cell strainer
6. Plunger of a 5-ml syringe
7. 6 well plates
8. T25, T75 and T150 cell culture flasks
9. Iscove's Modified Eagle's Medium (supplemented with 5% bovine serum albumin, 200 mM L-glutamine, penicillin/streptomycin, 50 μM 2-mercaptoethanol)
10. $OVA_{257-264}$ peptide (SIINFEKL)
11. Recombinant human interleukin-2 (see **Note 1**)
12. Optional: Red blood cell lysis buffer (0.15M NH_4Cl, 8.29 g/L; 10 mM $KHCO_3$, 1 g / L, EDTA 0.1 mM, 0.037 g/L in 1 l H_20; sterile-filtered with 0.22 μm filter, stored at 4°C)

2.2 CTL re-stimulation

1. Centrifuge
2. Cell culture incubator (37°C / 5% CO_2)
3. 15 ml or 50 ml Falcon tubes
4. 48 well or 24 well cell culture plates
5. Iscove's Modified Eagle's Media (supplemented with 5% bovine serum albumin, 200 mM L-glutamine, penicillin/streptomycin, 50 μM 2-mercaptoethanol)
6. Brefeldin A solution
7. $OVA_{257-264}$ peptide and variants (e.g. SIINFEKL, SIITFEKL, SIIGFEKL)

2.3 Flow cytometry analysis

1. Refrigerated centrifuge
2. Fluorescent-activated cell sorter
3. Flow cytometry sample tubes
4. Cell fixation and permeabilization reagents (e.g. eBioscience FoxP3 transcription factor staining buffer set)

5. Antibodies against T cell surface antigens and cytokines (e.g. CD8β, CD25, CD44, IFNγ, TNF) coupled to fluorescent conjugates (e.g. PE-Cy7, PE, APC-Cy7, Alexa-Fluor-488, PerCP Cy5.5)

6. Live / dead discrimination dye (e.g. Thermofisher Live/dead aqua fixable dye)

7. PBS-0.5% bovine serum albumin (BSA)

2.4 ID8 ovarian cancer cell culture

1. Centrifuge

2. Cell culture incubator (37°C / 5% CO_2)

3. T75 and T150 cell culture flasks

4. ID8-OVA-firefly luciferase (fLuc) cell line

5. RPMI-1640 media (supplemented with 5% bovine serum albumin, 200mM L-glutamine, penicillin/streptomycin)

6. Trypsin-EDTA (0.05%) solution

6. Sterile PBS

2.5 In vivo ID8 cancer model

1. 1 ml syringes

2. 27 or 29 Gauge needles

3. Luciferin

4. Small animal optical imaging system (e.g. IVIS imaging system)

3 – Methods

3.1 In vitro activation and expansion of OT-I T cells

Key steps in protocol 3.1 are illustrated in Figure 1. [Fig. 1 near here]

1. Dissect lymph nodes ± spleen from *Rag1*$^{-/-}$ OT-I or *Rag1*$^{-/-}$ *Ptpn22*$^{-/-}$ OT-I mice (see **Note 2**), being careful to avoid excessive amounts of fat, and transfer to 15 ml Falcon tube containing IMDM medium (see **Note 3**).

2. (Work is performed in a biosafety / tissue culture hood). Disrupt tissues using the plunger from a 5 ml syringe. Wash disrupted tissues with IMDM through a 40 μM cell filter placed on a 50 ml Falcon tube to create a single-cell suspension.

3. Pellet cells by centrifugation at room temperature (400 *g* / 5 min) and discard supernatant.

4. Optional: lyse red blood cells by resuspending cells in red blood cell lysis buffer for 2 minutes. Wash in fresh medium.

5. Resuspend cells in fresh IMDM and count using haemocytometer or cell counter.

6. Adjust cell concentration to 1.5 x 10^6 / ml for lymph node cells or 2 x 10^6 / ml for spleen cells.

7. Culture cells in 6 well plates, T25 or T75 flasks in presence of 10^{-9} M $OVA_{257-264}$ (SIINFEKL) peptide in cell culture incubator at 37°C / 5% CO_2.

8. Following 2 days of culture, transfer cell suspension to 15ml Falcon tube(s). Add fresh IMDM to a final volume of 12 ml and pellet by centrifugation at room temperature (400 *g* / 5min).

9. Resuspend pellet and repeat cell wash in fresh IMDM, to remove excess $OVA_{257-264}$ peptide. Pellet by centrifugation as described above.

10. Resuspend cells in fresh IMDM and count using haemocytometer or cell counter. Adjust cell concentration to 2 x 10^5 / ml.

11. Culture OT-I T cells in T75 or T150 cell culture flasks, as appropriate, in presence of 20 ng / ml recombinant human IL-2 in incubator at 37°C / 5% CO_2 (see **Notes 1, 4**).

12. Following a further 2 days of culture, repeat steps 8-11.

13. By day 6 of culture, OT-I T cells will have differentiated to an effector CTL phenotype (see **Note 5**) and can be used for *in vitro* (see 3.2) and *in vivo* (see 3.5) functional analyses.

3.2 Re-stimulation of effector CTLs

1. (Work is performed in a biosafety / tissue culture hood). Transfer day 6 OT-I CTL cell suspensions to 15 or 50 ml Falcon tubes. Pellet by centrifugation at room temperature (400 *g* / 5 min).

2. Resuspend cells in fresh IMDM and count using haemocytometer or cell counter. Adjust cell concentration to 1 x 10^6 / ml.

3. Add Brefeldin A (see **Note 6**) to cells, at a final concentration of 2.5 μg / ml, to prevent release of cytokines.

4. Stimulate 5 x 10^5 OT-I CTL in 48 well or 24 well plates with a titration of $OVA_{257-264}$ and variant peptides, typically 10-fold dilutions from 10^{-6} – 10^{-10} M, for 4 hours in a cell culture incubator (37°C / 5% CO_2) (see **Note 7**). For both wild-type and *Ptpn22*$^{-/-}$ cells, additional control samples should be left unstimulated (see **Note 8**).

3.3 Cell staining and flow cytometry analysis

1. Transfer 5 x 10^5 control and stimulated OT-I CTLs (see 3.2) to flow cytometry tubes (see **Note 9**) and wash in PBS-0.5% BSA. Pellet cells by centrifugation at 4°C.

2. Prepare master-mix of fluorophore-conjugated antibodies for self-surface antigens and live/dead dye by diluting in chilled PBS-0.5% BSA. Prepare 100 μl of antibody master-mix per sample plus an additional 100-200 μl, to ensure sufficient volume for all samples. Antibody dilutions are typically in the region of 1:100-1:1000, but should be titrated for each antibody/fluorophore.

3. To enable compensation to be set on the flow cytometer or during data analysis, additional control samples should be stained as single-colour controls for each antibody-fluorophore combination. For this purpose, resuspend cells in 100 μl of PBS-BSA containing one antibody-conjugate or live/dead dye diluted to same concentration as for the master-mix (see step 2). A further essential control is an unstained sample whilst the use of isotype control antibodies and fluorescence minus one (FMO) stained controls should be considered.

4. Stain experimental cell samples and controls in 100 μl / tube for 20 minutes at 4°C in the dark.

5. Wash cells by adding 1-2 ml PBS per tube. Pellet samples by centrifugation at 4°C (400 *g* / 5 min).

6. Fix and permeabilise samples using manufacturer's instructions for kit of choice. If using the eBioscience FoxP3 staining kit, fix and permeabilize samples in 50 μl of fix/perm buffer for 20 minutes at 4°C, followed by washing in 1 ml chilled permeabilization buffer and pelleting by centrifugation at 4°C (400 *g* / 5 min).

7. Prepare master-mix of fluorophore-conjugated antibodies for intracellular antigens (e.g. cytokines) by diluting in chilled permeabilization buffer. Prepare 50 μl of antibody master-mix per sample plus an additional 100 μl, to ensure sufficient volume for all samples. As described above, antibody dilutions should be titrated for each antibody/fluorophore and single-colour controls should be prepared (See Step 3).

8. Stain experimental cell samples and controls in 50 μl / tube for 30 minutes at 4°C in the dark (See **Note 10**).

9. Wash cells by adding 1-2 ml permeabilization buffer per tube. Pellet samples by centrifugation at 4°C (400 *g* / 5 min).

10. Resuspend cells in 350 μl PBS-0.5% BSA. If clumps or debris are present, cell suspensions should be run through a 40 μm cell strainer prior to analysis to prevent blocking cytometer tubing.

11. Using single-colour control and unstained samples, set compensation on flow cytometer. Analyze the experimental cell suspensions and collect at least 10^4 live cell events / sample.

3.4 Culture of ID8 ovarian carcinoma cells

1. (Work is performed in a biosafety / tissue culture hood). Upon receipt of frozen vials of ID8-OVA-fLuc cells (see **Note 11**), defrost by quickly warming in the hands and transfer cell suspension to a 15 ml Falcon.

2. Wash cells by adding 10 ml pre-warmed (37°C) complete RPMI-1640 (**Note 3**). Pellet by centrifugation at room temperature (400 *g* / 5 min).

3. Resuspend cells in 2 ml fresh RPMI-1640 and count using haemocytometer or cell counter. Adjust cell concentration to 1 x 10^6 / ml.

4. Transfer 5 x 10^5 or 1 x 10^6 cells to a T75 or T150 flask, respectively. Add RPMI-1640 to flasks in sufficient volume to cover the surface area of the flask bottom.

5. Growth of ID8 cells should be checked daily. Once adherent ID8 cells reach 70-80% confluency, remove RPMI-1640 using a vacuum pump or pipettor. Wash remaining RPMI-1640 from flask and remove non-adherent, dead cells by adding 10 ml pre-warmed sterile PBS and remove using a pipettor.

6. Detach adherent cells by adding 0.5 ml or 1 ml Trypsin-0.05% EDTA per T75 or T150 flask, respectively. Incubate for up to 5 minutes at 37°C, checking halfway through to determine if cells have begun to detach. Add 10 ml RPMI-1640, mix thoroughly use a pipettor to ensure all cells are resuspended, and transfer to 15 ml Falcon tube.

7. Pellet by centrifugation at room temperature (400 *g* / 5 min).

8. If continued propagation of cells is required, repeat steps 3-7. If cells are required for *in vivo* experiments, re-suspend in sterile PBS and count using haemocytometer or cell counter. Repeat wash step using sterile PBS and pellet by centrifugation.

9. Prepare cells for injection by re-suspending at 5 x 10^7 / ml in sterile PBS and retain on ice.

3.5 In vivo ID8 cancer model

Key steps in the *in vivo* ID8 cancer model are illustrated in Figure 2. [Fig. 2 near here]

1. In the animal facility, inject 100 μl of ID8 cell suspension intraperitoneally to recipient female C57BL/6 mice (5 x 10^6 cells / mouse) (see **Note 12**).

2. At least 7 days after ID8 cell injection, tumor burden should be assessed in mice by injecting luciferin intraperitoneally and performing intravital imaging under general anaesthetic (see **Note 13**). Mice are assigned to experimental groups, ensuring that all groups have similar average and ranges of tumour burden, as determined by analysis of luciferase activity, prior to treatment.

3. For preparation of wild-type and *Ptpn22*$^{-/-}$ OT-I CTLs for adoptive T cell transfer, follow steps 3.1 1-10.

4. Inject wild-type or *Ptpn22*$^{-/-}$ OT-I CTLs intravenously to recipient mice (see **Note 14**). A third control group should receive a mock adoptive cell transfer (PBS alone).

5. The impact of OT-I cell adoptive cell transfer on tumor growth is assessed by repeated luciferin injection and intravital imaging of luciferase activity at multiple timepoints post injection (see **Note 15**).

6. At experimental endpoints, mice are sacrificed. *Ex vivo* analysis (e.g. flow cytometric analysis) of transferred OT-I T cell population present in peritoneal washes can be performed if required (see **Note 16**).

4 – Notes

1. The use of human IL-2 is recommended, however recombinant mouse IL-2 is a suitable replacement. Concentrations of mouse IL-2 to be used in cell culture must be titrated by individual researchers.

2. Our control and *Ptpn22*$^{-/-}$ OT-I mouse lines have been backcrossed to a *Rag1*$^{-/-}$ background. This ensures that development of B cells and non-TCR transgenic T cells is blocked. As a consequence, OT-I T cells represent >90% of all cells in lymph nodes, but are less pure in spleens due to high proportions of myeloid cells. It is anticipated that the described cell culture and activation conditions will also be applicable or can be easily adapted to OT-I mouse strains on a *Rag1*-sufficient background.

3. The use of IMDM for culture of OT-I T cells is recommended, however alternative media preparations such as RPMI-1640 may serve as an alternative. ID8 cells will grow well in most common media preparations including IMDM, RPMI-1640 and DMEM. Of note, irrespective of media choice, it is essential to include 2-mercaptoethanol in culture to sustain primary T cell proliferation, but not ID8 cells.

4. Control OT-I CTLs grown under the described culture conditions expand exponentially between days 2-6. Large cell clumps will be visible by eye during this time and are a normal feature of highly proliferative T cells in culture. Researchers should expect at least 10-fold expansion between days 2 and 4. IMDM may “turn yellow” due to rapid OT-I cell proliferation, nutrient consumption and lactate secretion; transfer cells promptly to fresh medium and IL-2 when this happens. When cell clumps begin to fall apart, this is a sign that nutrients and / or IL-2 are running low. Ideally, researchers should check OT-I cell cultures daily to ensure that growth is optimal.

5. The protocol given here generates highly inflammatory effector CTLs that have potent anti-tumor responses but are relatively short-lived *in vivo* [8]. To generate longer-lived, memory phenotype CTLs, researchers should include soluble anti-CD28 antibody (1 μg / ml) during days 0-2 of culture, and replace human IL-2 with recombinant mouse IL-15 during days 4-6 of the culture period.

6. For intracellular cytokine staining, the use of Brefeldin A is recommended but the use of other protein transport inhibitors may also be applicable (e.g. monensin / golgi-stop).

7. It is strongly recommended that, for CTL re-stimulation experiments, researchers test recall responses to several different ovalbumin peptides of varying affinity for the OT-I TCR (e.g. SIINFEKL (high affinity), SIITFEKL (intermediate affinity), SIIGFEKL (low affinity) [12]) at a number of different concentrations. Our studies have shown that *Ptpn22*$^{-/-}$ OT-I CTLs have enhanced capacity to secrete cytokines in responses to low affinity but not high affinity antigens, as compared to *Ptpn22*$^{+/+}$

controls [6]. The use of several ovalbumin variant peptides therefore maximises the opportunity to distinguish differences between wild-type and *Ptpn22*$^{-/-}$ OT-I CTL responses.

8. An alternative to intracellular cytokine staining is analysis of secreted cytokines by supernatant ELISA or multiplex reagents. In this case, OT-I CTL cultures are set up as described without the addition of Brefeldin A, supernatant collected at 24h and stored at -20°C / -70°C before ELISAs are performed.

9. The use of flow cytometry sample tubes has been suggested, however all staining steps can also be performed in 96 well V-bottom or U-bottom plates, with corresponding adjustment of antibody mix and washing buffer volumes.

10. Staining times of 20 minutes for surface markers and 30 minutes for intracellular markers have been found to be suitable and convenient for the basic cell surface marker panel and specific cytokines described here, but optimal staining times may vary for other markers. Of note, a recent methods paper has evaluated the efficacy of overnight staining and the use of lower antibody concentrations for flow cytometry analysis of intracellular proteins in particular [14]. The impact of reduced antibody concentrations and longer staining times should be determined for each antibody.

11. We have described culture conditions and the *in vivo* use of ID8 cells expressing ovalbumin and firefly luciferase (ID8-OVA-fLuc), generated in our lab [7]. Variants of this cell line that express ovalbumin variants of varying affinity for the OT-I TCR are available and are useful for testing OT-I T cell responses to lower affinity tumor-associated antigens (described in References [7, 8]). All methods described here are applicable for the use of those ID8 variant cell lines. Investigators should allow approximately 7 days for expansion of ID8 cells *in vitro* before commencing injections and *in vivo* tumor models

12. In order to replicate the sex-specific hormonal environment, female C57BL/6 mice should be used exclusively as recipients for the *in vivo* ovarian cancer models. We recommend the use of 5 x 10^6 ID8 cells to establish reproducible tumors in mice, but researchers may wish to perform pilot studies with a titration of cancer cell numbers (e.g. 0.1-1 x 10^7). Numbers of mice required to detect reproducible statistically significant differences in tumor luminescence between experimental groups should be determined by the use of pilot studies and power calculations e.g. using the NC3Rs Experimental Design Assistant (https://eda.nc3rs.org.uk/experimental-design-group) or similar. The researcher performing cell injections and intravital imaging should be "blinded" as to which group receives the various treatments.

13. When using C57BL/6 mice as ID8 tumor cell-recipients, we recommend shaving the abdomen prior to, and in order improve the quality of, intravital imaging.

14. For efficient clearance / control of established ID8 tumours, numbers of OT-I T cells transferred should be between 0.5 – 1 x 10^7 cells / mouse.

15. The ID8 tumor model is relatively slow growing with mice typically surviving until ~day 100 post tumor cell injection [15]. At late stages of disease, mice will present with bloody, malignant ascites in the peritoneal cavity and should be humanely culled. The presence of ascites is likely to impact upon accurate detection of luciferase activity in the abdomen/peritoneal cavity. Variants of the ID8 line in which tumor suppressor genes have been deleted (e.g. *Tp53, Brca2*) have also been reported [16]. Deletion of tumor suppressor genes typically results in more aggressive and rapid *in vivo* tumor growth.

16. To enable identification and *ex vivo* analysis of OT-I T cells at experimental endpoints, the use of congenic mouse strains is recommended. Our control (*Ptpn22*$^{+/+}$) OT-I mouse strain expresses the CD45.1 allelic variant and therefore, T cells can be distinguished in adoptive cell transfer experiments on the basis of CD45.1 (donor) and CD45.2 (host) expression. For the same reasons, in some experiments, we have generated and used CD45.1/CD45.2 heterozygous *Ptpn22*$^{-/-}$ OT-I T cells [7]. Alternative approaches include the use of C57BL/6-Ubc-GFP transgenic mice, that have ubiquitous expression of GFP, as hosts [7]. In the absence of available transgenic or congenic mouse strains, the OT-I TCR can be identified using TCR Vα2 and Vβ5 antibodies, although a proportion of endogenous, host T cells will express the same TCR chains.

References

1. Bottini N, Peterson EJ (2014) Tyrosine phosphatase PTPN22: multifunctional regulator of immune signaling, development and disease. Annu Rev Immunol 32:83-119

2. Brownlie RJ, Zamoyska R, Salmond RJ (2018) Regulation of autoimmune and anti-tumour T-cell responses by PTPN22. Immunology 154:377-382

3. Mustelin T, Bottini N, Stanford SM (2019) The contribution of PTPN22 to rheumatic disease. Arthritis Rheumatol 71:486-495

4. Hasegawa K, Martin F, Huang G, Tumas D, Diehl D, Chan AC (2004) PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. Science 303:685-689

5. Brownlie RJ, Miosge LA, Vassilakos D, Svensson LM, Cope A, Zamoyska R (2012) Lack of the phosphatase PTPN22 increases adhesion of murine regulatory T cells to improve their immunosuppressive function. Sci Signal 5:ra87

6. Salmond RJ, Brownlie RJ, Morrison VL, Zamoyska R (2014) The tyrosine phosphatase PTPN22 discriminates weak self peptides from strong agonist TCR signals. Nat Immunol 15:875-883

7. Brownlie RJ, Garcia C, Ravasz M, Zehn D, Salmond RJ, Zamoyska R (2017) Resistance to TGFβ suppression and improved anti-tumor responses in $CD8^+$ T cells lacking PTPN22. Nat Commun 8:1343

8. Brownlie RJ,Wright D, Zamoyska R, Salmond RJ (2019) Deletion of PTPN22 improves effector and memory $CD8^+$ T cell responses to tumors. JCI Insight 5:e127847

9. Cubas R, Khan Z, Gong Q, Moskalenko M, Xiong H, Ou Q, Pai C, Rodriguez R, Cheung J, Chan AC (2020) Autoimmunity linked protein phosphatase PTPN22 as a target for cancer immunotherapy. J Immunother Cancer 8:e001439

10. Ho WJ, Croessmann S, Lin J, Phyo ZH, Charmsaz S, Danilova L, Mohan AA, Gross NE, Chen F, Dong J, Aggarwal D, Bai Y, Wang J, He J, Leatheman JM, Yarchoan M, Armstrong TD, Zaidi N, Fertig EJ, Denny JC, Park BH, Zhang ZY, Jaffee EM (2021) Systemic inhibition of PTPN22 augments anticancer immunity. J Clin Invest 131:e146950

11. Orozco RC, Marquardt K, Mowen K, Sherman LA (2021) Proautoimmune allele of tyrosine phosphatase, PTPN22, enhances tumor immunity. J Immunol 207:1662-1671

12. Hogquist KA, Jameson SC, Heath WR, Howard JL, Mevan MJ, Carbone FR (1994) T cell receptor antagonist peptides induce positive selection. Cell 76:17-27

13. Stepanek P, Prabhakar AS, Osswald C, King CG, Bulek A, Naeher D, Beaufils-Hugot M, Abanto ML, Galati V, Hausman B, Lang R, Cole DK, Huseby ES, Sewell AK, Chakraborty AK, Palmer E (2014) Coreceptor scanning by the T cell receptor provides a mechanism for T cell tolerance. Cell 159:333-345

14. Whyte CE, Tumes DJ, Liston A, Burton OT (2022) Do more with less: improving high parameter cytometry through overnight staining. Current Protocols 2:e589

15. Roby KF, Taylor CC, Sweetwood JP, Cheng Y, Pace JL, Tawfik O, Persons DL, Smith PG, Terranova PF (2000) Development of a syngeneic mouse model for events related to ovarian cancer. Carcinogenesis 21:585-591

16. Walton J, Blagih J, Ennis D, Leung E, Dowson S, Farquharson M, Tookman LA, Orange C, Athineos D, Mason S, Stevenson D, Blyth K, Strathdee D, Balkwill FR, Vousden K, Lockley M, McNeish IA (2016) CRISPR/Cas9-mediated Trp53 and Brca2 knockout to generate improved models of ovarian high-grade serous carcinoma. Cancer Res 76:6118-6129

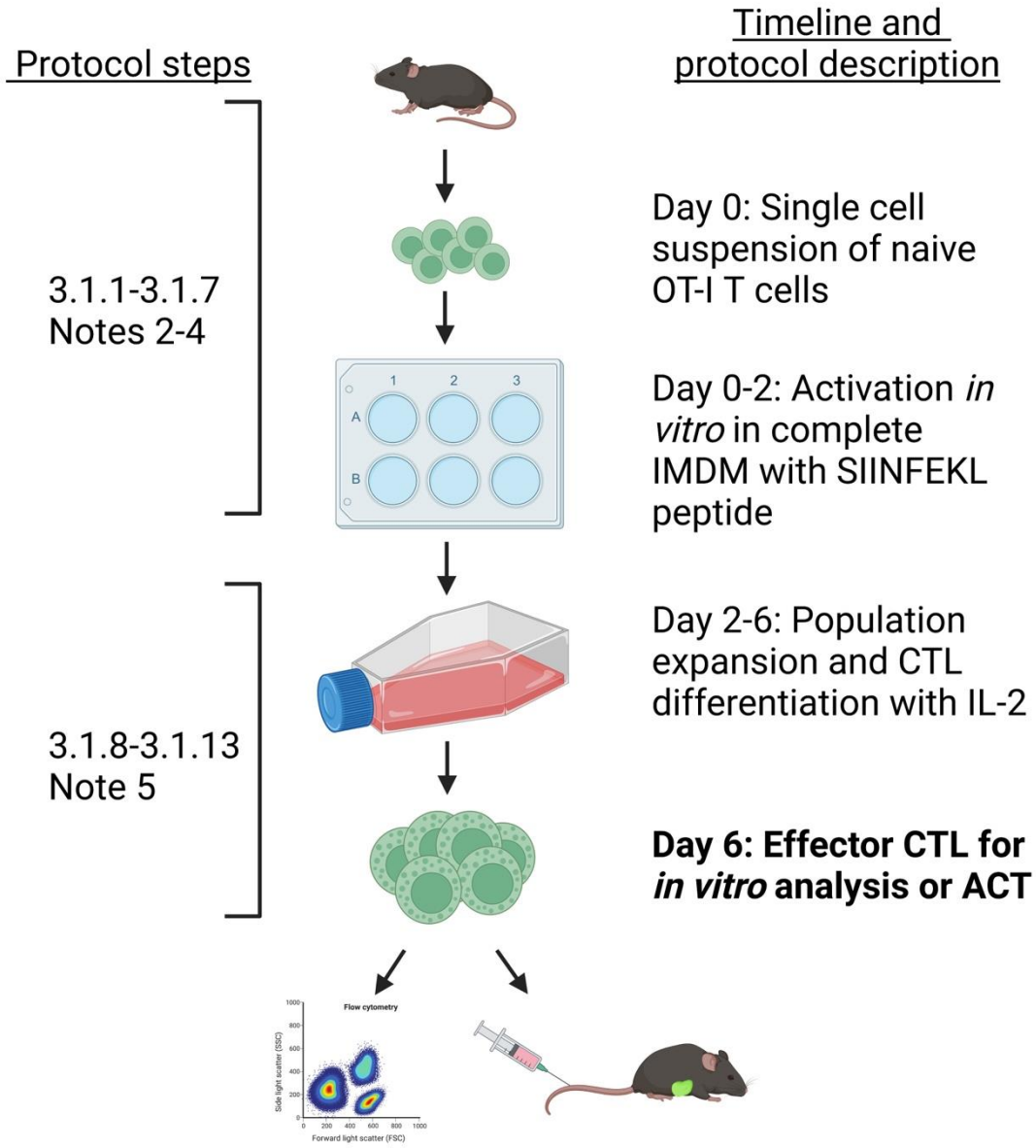

Figure 1. Overview of protocol steps for *in vitro* generation of effector OT-I CTLs. ACT – adoptive T cell transfer. Figure was created in Biorender.com.

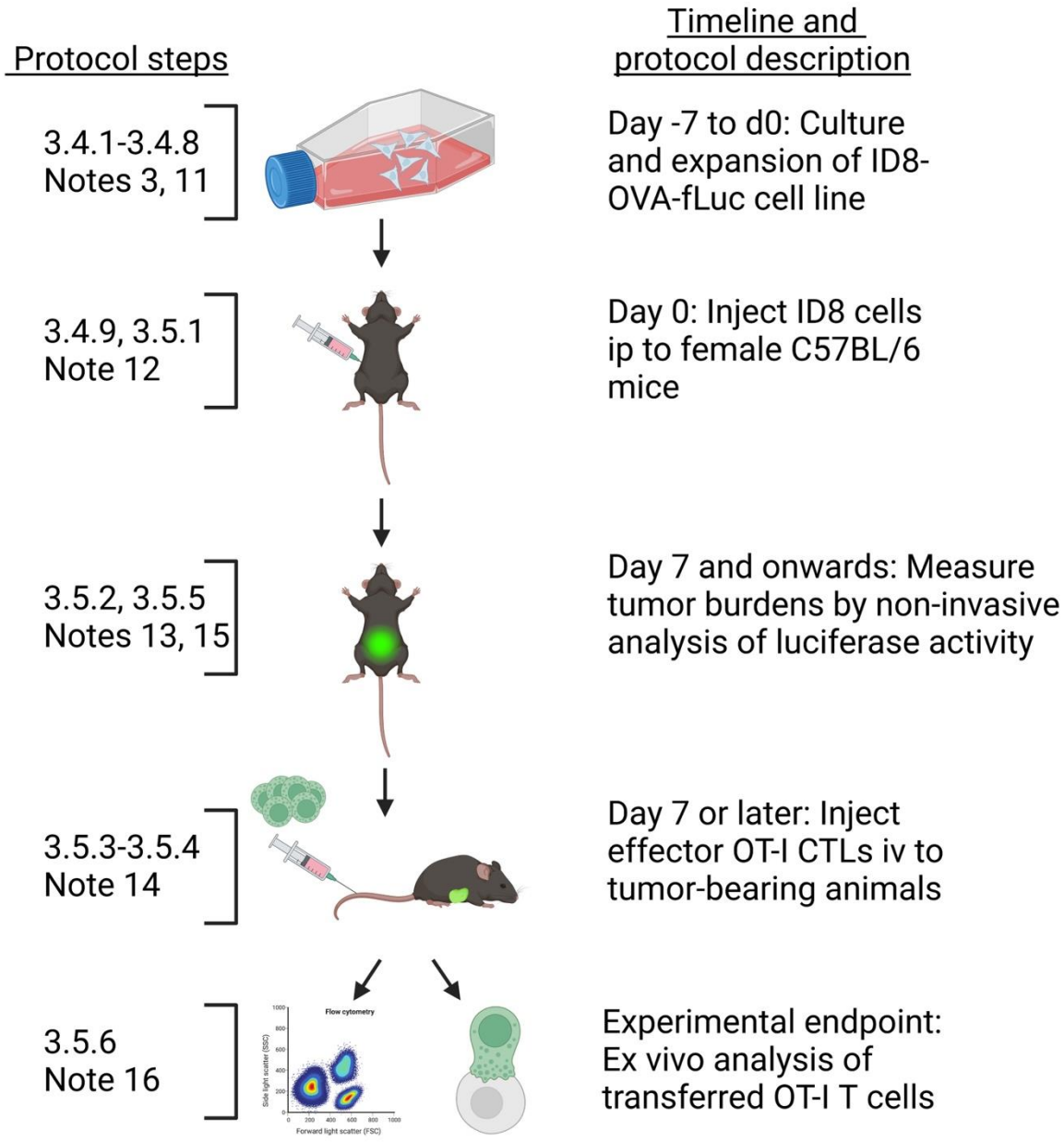

Figure 2. Overview of key protocol steps for assessment of *in vivo* OT-I CTL anti-tumor activity using ID8-OVA-fLuc ovarian carcinoma model. Figure was created in Biorender.com.