SELECTED OPPORTUNITIES IN IMMUNOTHERAPY

Ex-vivo active immunotherapy against infectious diseases and tumoral antigens for mimicking natural production by B cells of immunoglobulin

(BIO 15066)

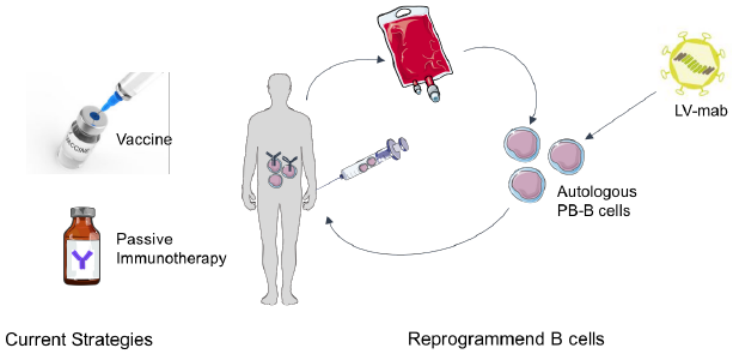
BIO15066

New method for reprogramming B cells with ectopic Ab-expressing constructs mimicking physiological regulation of Ab production

POC: HCV & HBV
Humanized mice

Techno

- **Method** : Adoptive transfer of B-cells transduced with a lentiviral vector (LV) conditionally expressing the secreted or the membrane-anchored form (BCR) of a transgenic immunoglobulin of interest, depending on the maturation status of transduced B-cells.



- **Product / Application**: Engineered B-cells producing an ectopic immunoglobulin of interest for *ex vivo* active immunotherapy, allowing long-term memory immune response.

- **POC**: 1) Hepatitis C virus: LV expressing the AR3A-IgG1 mAb (anti-HCV-E2 surface glycoprotein)
  - LV construct mimics the physiological expression of BCR and secreted Ab
  - Adoptive transfer of LV-transduced B-cells induces neutralizing Ab production *in vivo* in humanized mice.

  2) Hepatitis B virus: LV expressing three different antibodies against HBV induce functional neutralizing mAb *in vitro*.

- **Indications:** infectious diseases and cancer – ongoing project in cancer

- **Publication**: *Fusil F et al,* Mol Ther. 2015 Nov;23(11):1734-47.
- **Patent Application**: filed in 2015 - WO2017005923

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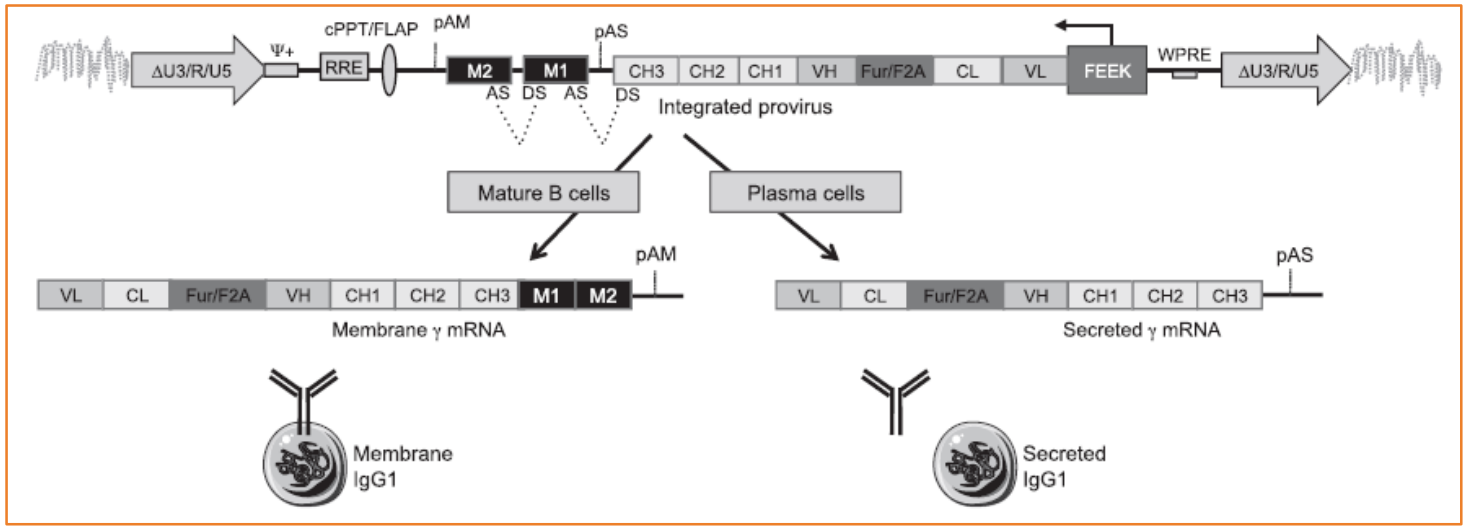
Transgene: FAM2 vectors conditionally expressing the membrane-anchored form of the AR3A Ab or the secreted form of the AR3A Ab depending on the maturation status of the B-cells.

FAM2 vector



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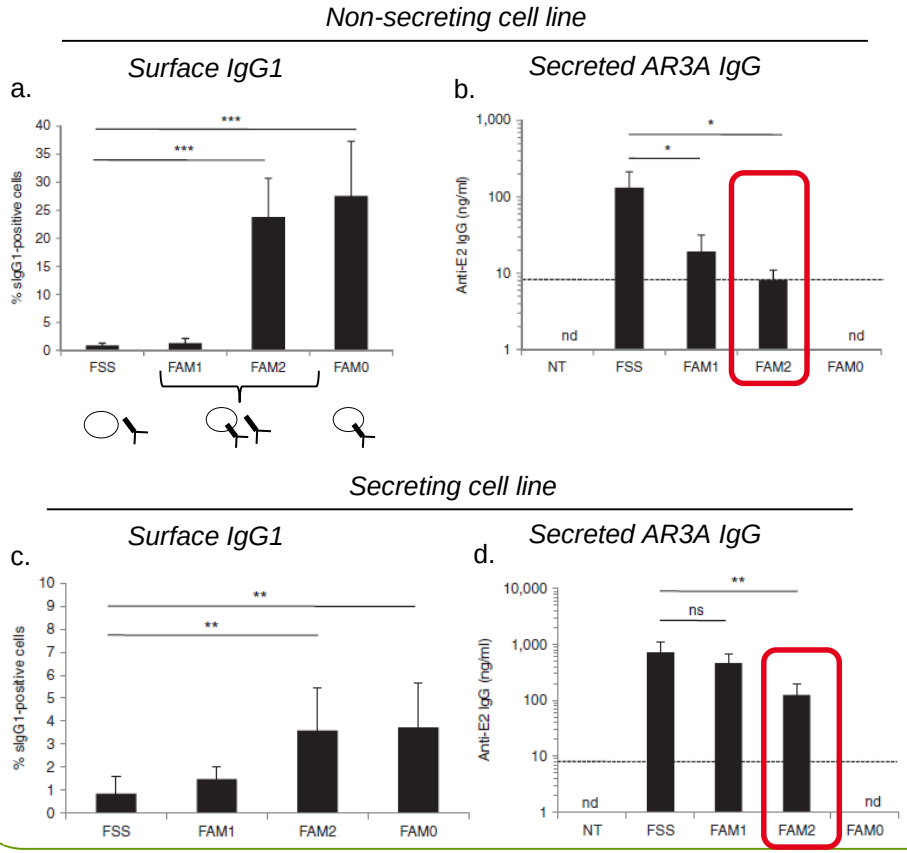
POC
In vitro

FAM2 LVs mediate expression of membrane-bound antibodies in a mature B-cell line and secreted antibodies in a plasmocytic B-cell line



Namalwa Burkitt Lymphoma (BL) cells (a non-secreting cell line) or human plasmocytoma U266 cells (a secreting cell line) were transduced with the indicated LVs. (a, c) The percentage of surface γ1 heavy chain (sIgG1) expressing cells were determined by flow cytometry analysis. (b, d) Levels of secreted anti-E2-specific IgGs in culture supernatants were quantified by specific anti-E2 enzyme-linked immunosorbent assay.

Controls:
FSS vector : expresses only the secreted form of the AR3A Ab
FAM0 vector: expresses only the membrane-anchored form of the AR3A Ab
FAM1 vector: FAM2 vector lacking the M1/M2 intronic sequence.

16-fold more secreted *AR3A* Ab in FAM2-transduced U266 secreting cells compared to BL non-secreting cells.

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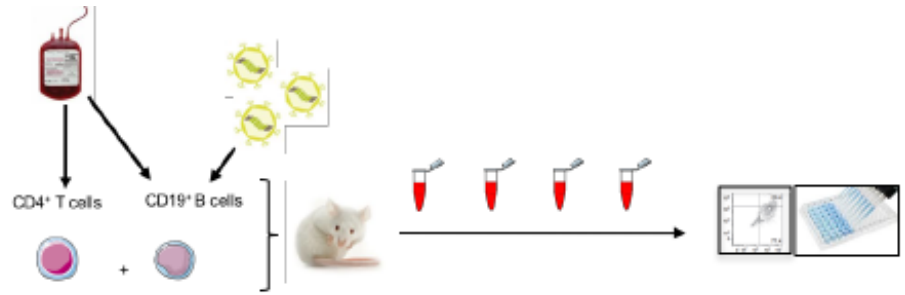
POC: HCV & HBV Humanized mice

POC
Adoptive transfer Humanized mice

Adoptive transfer of FAM2 LV-transduced B-cells induces secretion of neutralizing mAb *in vivo*

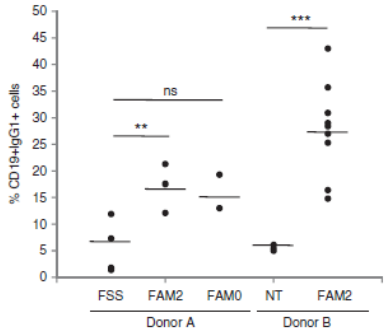
a.



***In vivo* expression of the transgenic AR3A antibody in humanized mice.** a) Purified human CD19+ B-cells were transduced with LVs. *In vivo* differentiation of these B-cells was induced by IP injection of the B-cells along with purified human CD4+ T cells into NSG mice. b) Splenic human B-cells (CD45+CD19+) were assessed for surface IgG1 expression (two different donors A and B). c) Percentage of anti E2 hIgG/human IgG in mouse sera was determined (d14: black bars, d21: gray bars). d) Neutralization assays of mouse sera were performed on Huh-7.5 cell line. HCVcc particles were incubated with mouse sera diluted at 1/20 for 1 hour at 37 °C before Huh-7.5 infection. Cells were washed 6 hours later and then cultured for 5 days. The "+" column: group of mice engrafted with CD19+ cells transduced with the AR3A-encoding vectors. The "-" column: group of control mice. The results show the % of neutralization of HCVcc by AR3A Ab.

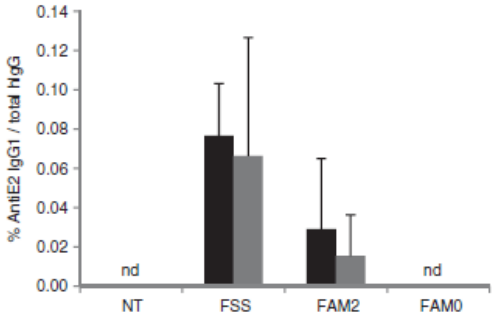
b.

Surface IgG1 in splenic B-cells



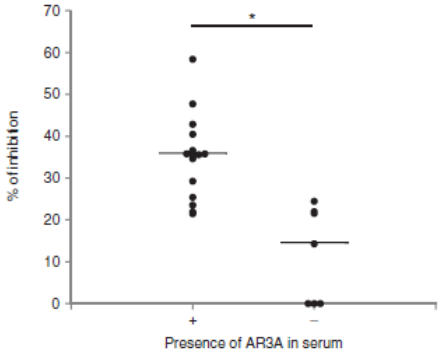
c.

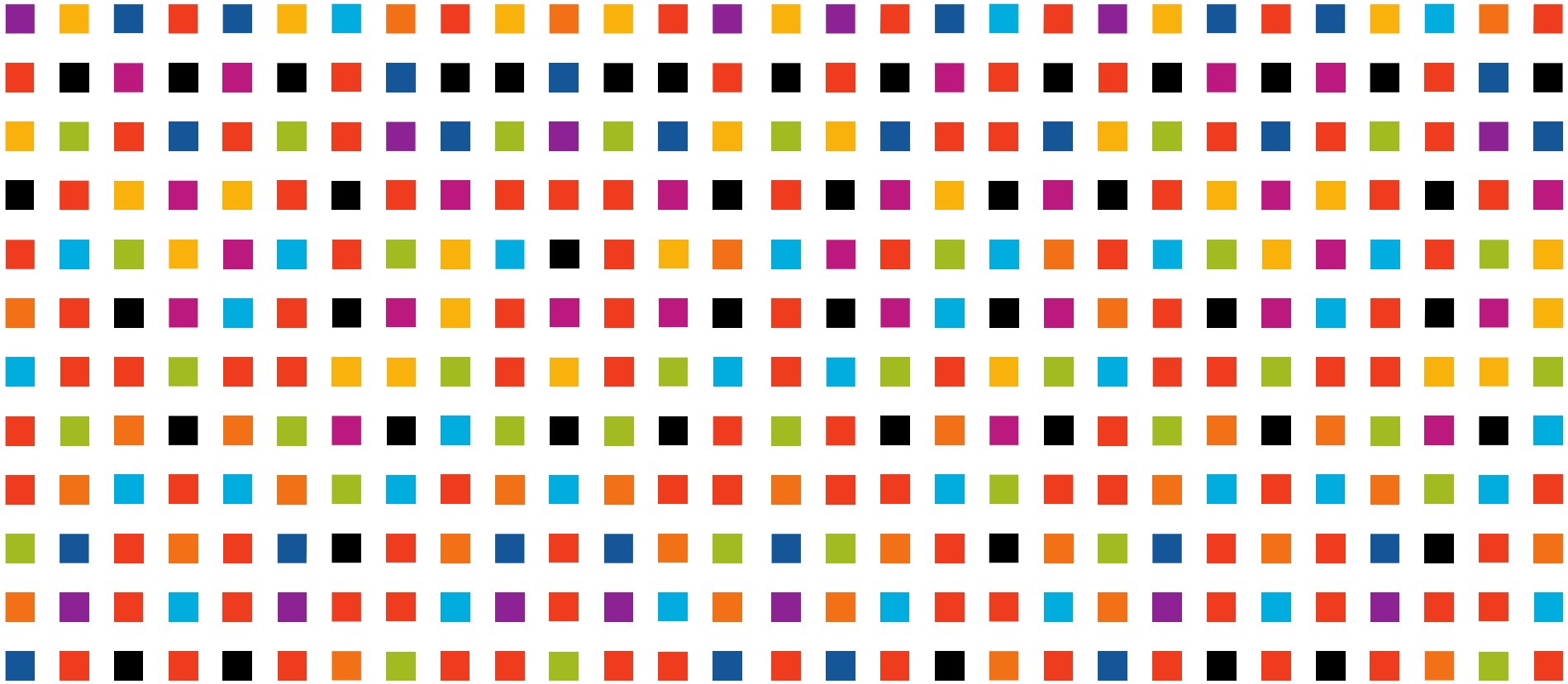
Secreted AR3A IgG in sera



d.

HCV neutralization assay





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