

Centre for Commercialization of **Regenerative** Medicine



BACKGROUND AND MOTIVATION

NK-92 cells exhibit cytotoxicity against a variety of cancer cell lines, including leukemia's, lymphoma's and malignant melanoma's. This activity has lead to several small clinical trials as an anti-cancer immunotherapy, and larger multi-centre Phase II trials are planned.





Current manufacturing strategy



Our GOAL is to enable large-scale manufacture of clinical grade NK-92 cells, and to reduce manufacturing costs. Production using perfusion culture in stirred-tank bioreactors (STR) would enable a straight-forward high productivity process that is readily scaled for commercial manufacture.



Figure 2. Effect of inoculum density and agitation rate in NK-92 cell expansion in STR. (A) Fold expansion and (B) <u>cell viability across different seeding densities and 4</u> agitation rates) using the ambr bioreactor system (TAP Biosystems). The greatest increase in viable cell number was observed when cells were seeded at 5 x 10⁵ cells/mL and stirred at 300-450 rpm.

6.0E+08 5.0E+08 XVivo 4.0E+08 3.0E+08 2.0E+08 1.0E+08 0.0E+00 XVivo-Static ---- DMEM-Statio

Figure 3. Effect of basal medium and culture system in NK-92 cell expansion. (A,B) Growth curves were obtained in static (T-75 flask) and stirred suspension (DasGip cellferm-pro) culture. Medium comprised Xvivo10 (Lonza) or DMEM (LifeTech) supplemented with human AB serum and L-glutamine, and cultures were initiated at a cell density of approx. 5 x 10⁵ cells/ mL. Culture volume was doubled every 2 days by addition of fresh media, up to a final volume 200 mL. (C) Specific growth rate (μ) was higher in STR compared to static culture in both medium formulations. (D) While NK-92's grow as loose clumps in static culture, STR culture yielded a single-cell suspension in Xvivo and smaller aggregates in DMEM. Scale bars = 100µm.



Figure 4. Effect of feeding-regime and culture system in NK-92 cell expansion. (A) Growth curves were obtained in 100 mL of stirred suspension culture (DasGip cellferm-pro). A 7.0-fold increase in cell numbers was observed when (B) a perfusion-like feeding-regime (1.0V) was adopted in the STR. (C) Metabolite analysis indicate that accumulation of lactate levels above 25 mM are inhibitory to cell growth.

DEVELOPMENT OF AN INTEGRATED BIOPROCESS FOR PRODUCTION OF NK-92 CELLS FOR IMMUNOTHERAPY

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FED-BATCH CULTURE





1.5E+06

1.3E+06

1.0E+06

7.5E+05

5.0E+05

2.5E+05

Xvivo



NIKON CORPORATION Instruments Company



CARBON SOURCE LACTATE in si si si si si so si so si so si si Glucose Xvivo + 2.5% BIT DMEM