NOVEL PHYTOCHEMICALS-BASED MEDIA (ADDED AS A SUPPLEMENT) IMPROVES THE EXHAUSTION AND ACTIVATION PHENOTYPE OF NK CELLS CULTURED IN COMMERCIALY AVAILABLE NK CELL MEDIA

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Background Natural killer cells, when cultured in specialized commercially available media for expanding NK cells, exhibit phenotypic changes (e.g. upregulation of exhaustion markers) that reduce their anti-cancer cytotoxic performance. The novel phytochemicals-based media Enable-NK™ developed to enhance NK cell proliferation and cytotoxicity, was used as a ‘supplement’ (10x concentration compared to the ready-to-use media version) and added to several commercially available media intended for NK cell culture, in order to test its additive effects.

Methods The human NK cell line KHYG-1 was cultured for 8 days in 24-well G-Rex plates, in either DMEM/F12 or a specific commercially available medium, with or without 10x Enable-NK™ supplement added (n=6 wells per condition). These four conditions were each tested at two different IL-2 concentrations, 100 and 500 U/mL (the manufacturer of the commercially available medium recommends 500 U/mL), all with 10% fetal bovine serum.

Results After 8 days in culture, Enable-NK™ supplemented groups demonstrated equivalent or better performance compared to their un-supplemented counterparts in terms of both proliferation and viability. Flow cytometry data illustrated that the percent of PD-1-expressing cells was low in DMEM/F12 with or without Enable-NK™ supplement (<2% PD-1+); in comparison, the PD-1+ percentage was significantly higher in the commercial NK medium (500 U/mL IL-2) at 8.8% PD-1+ cells. Addition of Enable-NK supplement to the commercially available medium reduced this percentage to 3.2% PD-1+ (p<0.0001). Addition of Enable-NK™ supplement also decreased the expression levels of the exhaustion markers NKG2A (p<0.0001), TIGIT (p=0.0002), and Lag-3 (p=0.0018), while increasing expression of the activation marker NKG2D (p<0.0001).

Conclusions Use of phytochemicals-based Enable-NK™ 10x supplement with commercially available media intended for NK cell culture reduces the development of an exhaustion phenotype, i.e. reduces the expression of inhibitory cell-surface receptors on NK cells cultured in vitro. Such supplementation could find use in both research and clinical settings, for example to mitigate inhibitory receptor expression during NK cell expansion for adoptive cellular therapy.

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