periférica. No decorrer do estudo 207 ICS foram diagnósticas, sendo 62% por bactérias Gram negativas (GN), 32% por Gram positivas (GP) e 6% por fungos. Em 33 (16%) das ICS a etiologia foi polimicrobiana. Nas três fases pós Alo TCTH, houve predomínio de GN em relação a GP. Os patógenos mais frequentes foram: *K pneumoniae* (n = 47), *S epidermides* (n = 41), *E coli* (n = 24), e *P. aeruginosa* (n = 16). Em relação a susceptibilidade dos GN, produção de betalactamase de espectro estendido (ESBL) foi identificada em 40% das *K. pneumoniae* e em 32% das *E. coli*, e produção de carbapenemase (ERC) ocorreu em 30% das *K. pneumoniae* isoladas. As incidências acumuladas de ICS por patógeno MDR no D+30, entre D+30 e D+100 e após o D+100 foram: 6%, 9% e 15% por GN produtores de ESBL e 3%, 5% e 7% por GN ERC. Em relação a ICS por GP, ocorreram 8 casos de ICS por *S. aureus, s* endo 3 (37%) resistentes a meticilina, e 13 ICS por enterococo, com 2 casos de resistência a vancomicina (15% das amostras). As incidências de ICS por MRSA e VRE foram de 1% e 2% na coorte, respectivamente. Em relação aos fatores de risco para ICS por MDR, a colonização previa por GN ERC estava presente em todos os casos que desenvolveram ICS por ERC ($p < 0,001$; VPP 12,6% e VPN 100%). Um paciente desenvolveu episódios recorrentes de ICS por ERC no decorrer do tratamento de segunda linha para DECH trato gastrointestinal não responsiva a corticosteroides. **Conclusão:** ICS foi um evento frequente nos pós Alo TCTH, com um predomínio de infecções por GN em todas as fases pós TCTH. Documentamos a emergência de MDR especialmente entre bactérias GN nas diferentes fases do pós Alo TCTH. Medidas para identificação precoce e tratamento antimicrobiano adequado são necessárias para conter a disseminação destes padrões de resistência e reduzir o impacto negativo destas infecções.

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NK-SPECIFIC CAR DESIGN IMPROVES NK CYTOTOXICITY AGAINST TUMOR CELLS

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Objectives: In recent years, studies with natural killer cells expressing CAR (CAR-NK) for cancer treatment have shown exciting results. Nevertheless, more studies focusing on the development of specific CAR molecules for NK cells are needed to generate CAR-NK cells with greater capacity for proliferation and activation and consequently improving their therapeutic efficacy. The aim of this study is to develop new combinations of CAR transmembrane region and intracellular domains to enhance CAR-NK signaling and cytotoxicity functions. **Methods:** Eight lentiviral vectors containing IL-15 secreting CARs composed of an extracellular single-chain variable fragment (scFv) anti-CD19 followed by different combination of transmembrane region, activator receptors and co-receptors were designed. Four of them were constituted by CD8 transmembrane region in combination with CD3z, DAP12, 4-1BB and/or 2B4: CAR19-CD8-41BB-CD3z (Insert A), CAR19-CD8-41BB-DAP12 (Insert B), CAR19-CD8-2B4-CD3z (Insert C), CAR19-CD8-2B4-DAP12 (Insert D). The other 4 vectors were composed of NKG2D transmembrane region in combination with CD3z, DAP12, 4-1BB and/or 2B4: CAR19-NKG2D-41BB-CD3z (Insert E), CAR19-NKG2D-41BB-DAP12 (Insert F), CAR19-NKG2D-2B4-CD3z (Insert G), CAR19- NKG2D -2B4-DAP12 (Insert H). Then, NK92 cell line was transduced, $CAR19^+$ cells were enriched using magnetic beads and $CAR19^+$ expression on cell surface evaluated by flow cytometry. Lastly, we evaluated *in vitro* potential of CAR-NK92 cells to kill Raji $CD19^+$ cancer cell lines stained with PKH67 cell linker at E:T ratio of 10:1 after 5 hours co-cultivation. **Results and discussion:** The expression of CARs containing CD8 transmembrane region (Insert A, B, C and D) on cell surface ranged from 2-4% CAR on day 7 post-transduction and remained stable until 21 days post-transduction and, after two selections, NK92 cells expressing 70-90% of CAR were obtained. The expression of CARs containing NKG2D transmembrane region (Insert E, F, G and H) ranged from 0.15-0.38% on day 7 post-transduction, and it was unfeasible to select these cells. Next, the cytotoxicity of NK92-CAR cells containing CD8 transmembrane region was evaluated *in vitro*. All NK92 cells containing CAR were more efficient in killing target cells than NK92WT cells (% Cytotoxicity rate mean±SD: -1.80±6.32). In addition, NK92-CAR Insert B (68.53±3.72), C (87±2.77) and D (65.18±2.12) cells were more cytotoxic than NK92-CAR Insert A cells (42.22±7.23; $p < 0.05$), being the NK92-CAR Insert C cells the most cytotoxic of all CARs at 10:1 ratio ($p < 0.05$). It is important to note that CAR-Insert A is the CAR commonly used in CAR-T cell therapy, suggesting that the vector containing receptors and co-receptors specific for NK cells was able to improve their function. **Conclusion:** Our results demonstrated that the transmembrane region affected CAR expression on cell surface, being the CD8 region more effective. Furthermore, NK92 cells expressing CAR specific for NK cells were more efficient in killing tumor target cells, and the NK92-CAR cell constituted by 2B4 and CD3z intracellular domain (Insert C) was especially effective. The therapeutic effectiveness of these cells will be evaluated *in vivo*. **Funding:** Financially supported by FAPESP 2020/08279-8, FAPESP 2019/25309-0, CTC Center for Cell-based Therapy (FAPESP 2013/08135-2), and National Institute of Science and Technology in Stem Cell and Cell Therapy (CNPq 573754-2008-0 and FAPESP 2008/578773).

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