P57 – **Cancer immunotherapy of TLR4 agonist-antigen constructs enhanced with pathogen-mimicking magnetite nanoparticles and checkpoint blockade of PD-L1**

Giordano Traini,[2] Ane Ruiz-de-Angulo,[2] and Juan C. Mareque-Rivas[1]*

[1]*Department of Chemistry and Centre for NanoHealth Swansea University, Singleton Park, Swansea, SA2 8PP, U.K.*

[2]*CIC biomaGUNE Paseo Miramón 182, 20014 San Sebastián, Spain*

traini.giordano@gmail.com

Despite the tremendous potential of Toll-like receptor (TLR) 4 agonists in cancer immunotherapy, only some lipopolysaccharides (LPS) isolated from particular bacterial strains or synthetic structures like monophosphoryl lipid A (MPLA) are able to avoid toxic overactivation of the immune system while retaining adequate immunogenicity to act as vaccine adjuvants[1]. For cancer immunotherapy applications it may be possible to improve TLR4 agonists by better delivery and immunostimulatory activity modulation using nanoparticles. Moreover, combination of vaccines with immune checkpoint blockade (CPB) strategies could overcome the intrinsic weaknesses of vaccines and CPB monotherapies[2].

Here, we developed a nanovaccine by incorporating through a self-assembly process different LPS-related structures into nanoparticle-filled phospholipid micelles (mNPs), exploiting the amphipathic structures of the adjuvants creating a nano-adjuvant for efficient vaccine delivery and potent cancer immunotherapy. The structurally unique LOS of the plant pathogen *Xcc* was incorporated into phospholipid micelles encapsulating oleic acid-coated 6 nm iron oxide nanoparticles (mIONPsp-*Xcc* LOS), producing stable pathogen-mimicking mNPs with ideal size, charge and hydrophobicity for targeting antigen presenting cells in the lymph nodes. The antigen OVA was attached to mIONPsp via a hydrazone bond (mIONPsp-HyNic-OVA) creating a nano-antigen and enabling rapid, easy-to-monitor and high-yield antigen ligation at low concentrations[3].

The protective effect of mIONPsp-HyNic-OVA formulated with mIONPsp-*Xcc* LOS as adjuvant was investigated in mice against the highly *aggressive* model for murine tumor immunotherapy, B16-F10 melanoma expressing OVA. The results showed that the nanovaccines led to a higher-level tumor antigen-specific cytotoxic T lymphocyte (*CTL*) effector and memory responses without inducing toxicity, and that when combined with abrogation of the immunosuppressive programmed death-ligand 1 (PD-L1), provided 100% long-term protection against repeated tumor challenge. The mIONPsp and antigen conjugation strategy in combination with immune checkpoint inhibition of PD-L1 represent a promising approach to improve cancer immunotherapy of vaccines exploiting TLR4 agonists.

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