SUPPLEMENTAL METHODS

FSHCER design

The binding domain of the FSHCER was engineered following the design of Fan and Hendrickson (1). Briefly, the N-terminal domain corresponded to the FSH beta signal peptide, followed by the mature sequence of FSH beta (residues 1-110 in the mouse and 1 to 111 in the human), followed by a glycine-serine linker (GGGSGGGSGGGSGGG), followed by the mature sequence of FSH alpha (residues 1-96 in the mouse, 1-92 in the human). The covalently linked FSH hormone was followed by the murine or human versions of a CD8 hinge and transmembrane domain, the intracellular domain of 4-1BB and the intracellular domain of CD3z.

The final amino acid sequences resulted as follows:

Murine:

MMKLIQLCILFWCWRAICCHSCELTNITISVEKEECRFCISINTTWCAGYCYTRDLVYKD
PARPNTQKVCTFKELVYETVRLPGCARHSDSLYTYPVATECHCGKCDSDSTDCTVRGL
GPSYCSFSEMKEGGGSGGGSGGGSGGGDFIIQGCPECKLKENKYFSKLGAPIYQCMGCC
FSRAYPTPARSKKTMLVPKNITSEATCCVAKAFTKATVMGNARVENHTECHCSTCYYH
KSASTTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVA
LLLSLIITLICKWIRKKFPHIFKQPFKKTTGAAQEEDACSCRCPQEEEGGGGGYELRAKFS
RSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQQRRRNPQEGVYNA
LQKDKMAEAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR

Human:

MKTLQFFFLFCCWKAICCNSCELTNITIAIEKEECRFCISINTTWCAGYCYTRDLVYKDPA
RPKIQKTCTFKELVYETVRVPGCAHHADSLYTYPVATQCHCGKCDSDSTDCTVRGLGPS
YCSFGEMKEGGGSGGGSGGGSGGGAPDVQDCPECTLQENPFFSQPGAPILQCMGCCFSR
AYPTPLRSKKTMLVQKNVTSESTCCVAKSYNRVTVMGGFKVENHTACHCSTCYYHKS
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLL
SLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADA
PAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDK
MAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Immunoblot and immunohistochemistry

Determination of positive/negative signal was based, in the case of WB analysis, on the presence of a band in the expected MW, compared to the negative expression in carcinosarcomas, as determined by 2 independent observers.

For IHC, the presence of positive signal above the negative control (CAVO3 cells stained with 2^{nd} antibody) was also evaluated by 2 independent observers.

FSHCER treatment of patient derived xenografts

Human ovarian tumors FCCC-OC-16, Wistar-1 and Wistar-2 were implanted subcutaneously. Wistar-3 was implanted as a tumor fragment under the ovarian bursal. We followed tumor growth and proceded with intratumoral injection (intraperitoneal in the case of Wistar-3) of 10 million T-cells when the tumors reached an average size of 150-200 mm^3. Five days later we

repeated the treatment in the cases of Wistar-1 and Wistar-2 because no obvious effect was evidenced. The experiments were terminated after signs of animal discomfort were evidenced due to an excesive tumor growth or signs of graft versus host disease.

Determination of endogenous anti-tumor response

CD45.2^{+} mice bearing ID8-*Defb29/Vegf-a*/*Fshr* syngeneic tumors were treated with either CAR T-cells (1.5e^{6} CD45.1^{+}cells intraperitoneally), mock-transduced T-cells (with retrovirus carrying the same plasmid as FSHCER but without the CER construct, also sorted on GFP, 1.5e^{6} CD45.1^{+}cells intraperitoneally) or PBS. One week later we FACS-sorted endogenous (CD45.2^{+}CD45.1^{-}GFP^{-}) T-cells obtained through peritoneal wash and analyzed their antitumor activity using interferon-gamma ELISPOT. For the ELISPOT we incubated the endogenous CD45.2^{+}CD45.1^{-}GFP^{-} T-cells from the different groups with bone marrow derived dendritic cells (BMDCs) obtained from a naïve mouse generated as previously described (2-4) pulsed with double gamma (10000 rad) and UV (30 minutes)-irradiated ID8-*Defb29/Vegf-a*/*Fshr*. 72 hours after the co-culture of the T-cells with the pulsed BMDCs we added the detection antibody and after incubating overnight at 4ºC we developed the ELISPOT and counted the spots using Immunospot software (CTL).

For the determination of the in vivo relevance of this increase in anti-tumor response from the endogenous T-cells of the adoptive T-cell transfer, we then treated CD45.2^{+} mice bearing ID8-*Defb29/Vegf-a*/*Fshr* syngeneic tumors with either CAR T-cells (1.5e^{6} CD45.1^{+}cells intraperitoneally) or mock-transduced T-cells (1.5e^{6} CD45.1^{+}cells intraperitoneally). One week later we FACS-sorted endogenous (CD45.2^{+}CD45.1^{-}GFP^{-}) T-cells from the spleen and injected

one million per mouse to sublethally irradiated mice. One day later we injected these mice in the flank with ID8-*Defb29/Vegf-a*/*Fshr* and monitored tumor growth by measuring tumor volume through time.

Flow cytometry instruments maintenance and calibration

Flow cytometry instruments are regularly maintained by The Wistar Institute Flow Cytometry Facility personnel. A long clean of the fluidics systems is done on a monthly basis. Frequent calibration of the optical system (laser delays and target voltages for PMTs) is performed using the BD Cytometer Setup and Tracking application and BD FACSDiva CS&T research beads.

REFERENCES

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3. Nesbeth YC, Martinez DG, Toraya S, Scarlett UK, Cubillos-Ruiz JR, Rutkowski MR, et al. CD4+ T cells elicit host immune responses to MHC class II- ovarian cancer through CCL5 secretion and CD40-mediated licensing of dendritic cells. J Immunol 2010;184(10):5654-62.

4. Rutkowski MR, Stephen TL, Svoronos N, Allegrezza MJ, Tesone AJ, Perales-Puchalt A, et al. Microbially driven TLR5-dependent signaling governs distal malignant progression through tumor-promoting inflammation. Cancer Cell 2015;27(1):27-40.