or hMSLNpos human mesothelioma cell line, MSTO-211H, or stimulated with anti-CD3/anti-CD28 antibodies in vitro for 8 days. Distinct cell populations in MCY-M11 were evaluated for kinetics and duration of CAR expression, differentiation, activation, exhaustion, and their ability to secrete various immunomodulatory molecules during in vitro stimulation. Antigen-specific proliferation and cytotoxicity of MCY-M11 against hMSLNpos tumor cells as well as their ability to mount long-term antitumor immunity through epitope spreading mechanisms were studied.

**Results** Individual cell populations in MCY-M11 exhibited a consistent but transient Meso-CAR expression persisting for about 7 days. Cell subsets in MCY-M11 acquired early signs of activation and differentiation within 18–24 hours post-culture, but only attained full activation and lineage-specific differentiation upon specific response to hMSLNpos tumor cells. hMSLN antigen experienced MCY-M11 retained significant fractions of naïve and central memory T cells and increased percentage of Effector memory T cells along with increased expression of CD62L, CD27, and chemokine receptors (CCR5, CCR7, and CXCR3). MCY-M11 exhibited strong antigen-specific cytotoxicity against hMSLNpos tumor cells with corresponding increase in activation and proliferation of CD4+ and CD8+ T cell subsets and displayed low or no acquisition of known exhaustion markers. NK cells also exhibited a functionally superior molecular signature exhibiting increased levels of NKG2D, NKp44, NKp46, FAS, and TRAIL. The Monocytes and B cells in MCY-M11 also acquired an activated, differentiated, and mature phenotype, expressing molecules required for antigen presentation (HLA-DR, HLA-ABC, and CD205) and T cell co-stimulation (CD80 and CD86) to mount a strong antitumor response. These phenotypic changes in cell subsets of MCY-M11 transpired with simultaneous secretion of potent immunostimulatory molecules and chemokines facilitating an extended antitumor response through epitope spreading.

**Conclusions** We demonstrated that MCY-M11 is a unique cell product possessing a complete built-in immune cellular machinery with favorable phenotype and enhanced functions specialized in mediating an effective and long-term antitumor response.

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