T memory stem cells (T<sub>SCM</sub>), a rare subset of memory lymphocytes endowed with enhanced capacity of self-renewal and the potential to reconstitute the full repertoire of memory and effector cells, have recently emerged as a central player in several physiological and pathological processes. T<sub>SCM</sub> cells have been shown to mediate superior anti-tumor responses (Gattinoni et al., 2009, Gattinoni et al., 2010), harbor leukemic cancer stem cells (Nagai et al., 2015), and serve as a reservoir for HIV infection (Buzon et al., 2014). Because of these wide-ranging clinical implications, it is of critical importance to improve our understanding of the molecular events that induce or maintain T<sub>SCM</sub> cells.

In this issue of EbioMedicine, Scholz et al. used pharmacological and genetic approaches to identify mTOR as a key signaling pathway regulating the formation of CD4<sup>+</sup> and CD8<sup>+</sup> T<sub>SCM</sub> cells (Scholz et al., 2016). It has previously been demonstrated in murine (Gattinoni et al., 2009) and human (Gattinoni et al., 2011) models that the formation of T<sub>SCM</sub> cells can be promoted by triggering WNT/β-catenin signaling. The finding that inhibition of the mTOR pathway can also favor the generation of T<sub>SCM</sub> Cells has significant therapeutic implications as rapamycin and other mTOR inhibitors are FDA-approved agents that have already successfully been employed to generate T<sub>SCM</sub> cells (Gattinoni et al., 2009, Muralidharan et al., 2011). In their experiments, the authors found that T<sub>SCM</sub> cells manufactured in rapamycin had increased persistence relative to control naïve or central-memory T cells, recapitulating prior observations obtained using naturally occurring T<sub>SCM</sub> cell populations (Gattinoni et al., 2011). These findings are also consistent with a body of literature indicating that rapamycin-resistant human Th1/Tc1 cells have a remarkable ability to repopulate xenogeneic hosts and mediate graft-versus-host disease effects (Amaranth et al., 2010). Additional in vivo functional endpoints, however, will be needed to determine the therapeutic potential of the rapamycin-generated T<sub>SCM</sub> cell populations as manufactured by Scholz et al.

There is a growing interest around the possibility of targeting metabolism for immunotherapeutic interventions as it has become clear that metabolism can profoundly influence T cell functionality and fate commitment. Up to this point, the metabolic profile of T<sub>SCM</sub> cells has remained elusive. Now, Scholz et al. shed new light on the metabolic regulation of human CD4<sup>+</sup> T<sub>SCM</sub> cells and have revealed that, similar to naïve and memory T cell populations, T<sub>SCM</sub> cells rely on fatty acid oxidation as a primary source for ATP synthesis. These findings are fundamental because they open up the possibility to promote the formation and maintenance of T<sub>SCM</sub> cells through the manipulation of fatty acid metabolism.

While underlying a central role for mTOR in the generation of T<sub>SCM</sub> cells, Scholz et al. dispute a role for WNT/β-catenin signaling. The authors argue that the WNT activator, TWS119, promoted T<sub>SCM</sub> cell generation not by the conventionally recognized mechanism (GSK3β inhibition and subsequent β-catenin stabilization) but rather via an off-target effect that involved mTOR inhibition; their conclusion was based in part on their observations that neither a physiological WNT3A ligand nor alternative GSK3β inhibitors supported T<sub>SCM</sub> formation in their hands. However, the authors used WNT3A at concentrations 100-fold lower than those used in previous studies that found an active role of WNT3A in the generation of both murine and human T<sub>SCM</sub> cells (Gattinoni et al., 2009, Muralidharan et al., 2011). In their experiments, the lack of activity of the GSK3β antagonist indirubin-3'-monoxide was potentially attributable to its weak selectivity and inability to trigger downstream WNT signals (Meijer et al., 2003). In sharp contrast, the highly selective, 6-bromoindirubin derivatives, which are capable of stabilizing β-catenin (Meijer et al., 2003), have successfully been employed to generate T<sub>SCM</sub> Cells (Gattinoni et al., 2009). It is also important to underscore that the authors did not provide evidence of the ability of these 'ineffective' agents to activate WNT/β-catenin signaling. Indeed, Scholz et al. employed as a WNT operational readout the phosphorylation of GSK3β/serine 9, a post-translational modification mediated by AKT and not involved in WNT signaling (McManus et al., 2005) (gold standard assays would consist of measurement of unphosphorylated β-catenin and WNT-reporter activity). In a final attempt to support their conclusion of TWS119 action outside of the GSK3β/WNT pathway realm, the authors used an elegant model involving β- and γ-catenin deficient T cells. However, conclusions based on these experiments carry the major caveat that WNT signal transmission is substantially maintained in double-deficient T cells (Jeannet et al., 2016).
et al., 2008). Further research will therefore be required to more definitively test the extent to which GSK3β inhibitors such as TWS119 might mediate their effect on TSCM cells independent of the WNT pathway.

In summary, Scholz et al. have tackled a critical issue in biomedicine relating to human CD4+ and CD8+ T memory stem cell generation and function. Although the molecular mechanism(s) of TSCM cell generation remain nebulous vis-à-vis the relative role of WNT/β-catenin or mTOR modulation, the current report certainly provides a nidus of information that will guide subsequent investigations. Hopefully, with the attainment of a refined molecular understanding and the development of alternative pharmacologic inhibitors, an ability to effectively modulate TSCM cells for therapeutic purposes will be realized.

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