**Abstracts**

**402 MITOPHAGY REINVIGORATES EXHAUSTED CD8 T CELL**

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**Background** Early exhaustion of tumor infiltrating CD8 T cells is a major obstacle that restricts the effectiveness of immunotherapy in a broader range of cancer patients. Mitochondrial health is a crucial factor determining the performance and endurance of CD8 T cells within the immunosuppressive tumor microenvironment (TME). Apart from encountering immunosuppressive signals, CD8 T cells also face metabolic challenges such as hypoxia and nutrient deprivation in the TME. Recent studies have shown promising results by employing strategies that enhance mitochondrial function to rejuvenate exhausted tumor-infiltrating lymphocytes (TILs).

Mitophagy, an intrinsic process for removing dysfunctional mitochondria, plays a critical role in maintaining mitochondrial quality and metabolic homeostasis. Inhibiting USP30, a mitochondrial deubiquitinase, has been demonstrated to enhance mitophagy and improve mitochondrial function. We propose that the inhibition of USP30 in CD8 T cells could enhance their performance and endurance within the TME by preserving mitochondrial function.

**Methods** In this study, we utilized mitophagy reporter (mt-Keima) mice to investigate the mitophagy activity of tumor-infiltrating CD8 T cells based on their exhaustion statuses. We genetically and pharmacologically inhibited USP30 in CD8 T cells and assessed their mitophagy activity, mitochondrial functions, T cell functions, and exhaustion levels within the TME. Additionally, we treated tumor xenograft mice with a USP30 inhibitor and adoptive CD8 T cells with USP30 deletion to evaluate the potential benefits of targeting USP30 in immunotherapies.

**Results** Our findings revealed that mitophagy activity, indicated by the mt-Keima fluorescence, is intrinsically reduced in activated CD8 T cells and further compromised in exhausted CD8 T cells expressing PD1 and TIM3 markers (figure 1). Inhibiting USP30 effectively increased mitophagy activity in exhausted CD8 T cells, thereby enhancing mitochondrial efficiency (figure 2). Furthermore, treating mice with a USP30 inhibitor or transferring USP30 knockout adoptive CD8 T cells resulted in reduced T cell exhaustion and improved CD8 T cell functionality, ultimately enhancing anti-tumor immunity (figure 3).

**Conclusions** Mitophagy plays a critical role in maintaining mitochondrial health in CD8 T cells within the TME. However, the exhaustion of CD8 T cells can result in the suppression of mitophagy and impaired mitochondrial function. By promoting mitophagy, exhausted CD8 T cells can be rejuvenated, leading to improved function and endurance within the TME. Targeting USP30 is a promising approach to promote mitophagy activity in CD8 T cells and develop more effective immunotherapy strategies.

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**REFERENCES**


**Abstract 402 Figure 1** (A, B) Mitophagy activity (Keima Red) in tumor infiltrated CD8 T cells based on exhaustion degree. (C, D) Mitochondrial membrane potential (TMRM) and mass (Mitotracker Green) in tumor infiltrated CD8 T cells based on exhaustion degree.

**Abstract 402 Figure 2** (A, B) Mitophagy activity (Keima Red) in exhausted CD8 T cells treated with vehicle or USP30 inhibitor in vitro. (C, D) Mitochondrial membrane potential (TMRM) and mass (Mitotracker Green) in exhausted CD8 T cells treated with vehicle or USP30 inhibitor in vitro.
Abstract 402 Figure 3  (A) tumor growth curve of xenograft mice treated with vehicle or USP30 inhibitor (ST-539). (B) tumor growth curve of xenograft mice with or without USP30 knockout on T cells (LCK CRE). (C,D,E) Exhaustion degree (PD1 & TIM3) of tumor infiltrated CD8 T cell with or without USP30 knockout.

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