Background
We have shown that malignant pleural effusions (MPE) are characterized by a distinct and complex pleural secretome dominated by IL-6, sIL-6R, CCL2, CXCL10, TGFβ, CCL22, IL-8. These cytokines favor tumor epithelial to mesenchymal transition, invasion and suppression of anti-tumor response among the abundant infiltrating T cells. The goal of this study was to determine whether brief ex vivo activation could induce potent effector activity.

Methods
Pleural T cells were isolated from freshly drained pleural effusions from 6 breast cancer patients. Autologous pleural tumor was expanded in vitro using the Mammary Epithelial Growth Medium (Lonza). Pleural T cells were stimulated using antiCD3CD28 Dynal beads and low dose IL-2 (60 Cetus U/ml) for 2, 4, 7, 14 or 21 days. Expanded tumor targets (p0 and p1) were plated at 10,000 cells/96well cultured overnight before the addition of pleural effector T cells at effector to target ratios of 0.1, 3, 6, 12.5, 25 and 50. Cytotoxicity was assessed using a colorimetric assay for lactate dehydrogenase (LDH) release (PromegaCytox-96). Pleural T cells phenotype and activation/exhaustion markers. Cytokine secretion was measured by flow cytometry and (Luminex).

Results
Ex vivo expanded pleural T cells were effector-memory phenotype (CD45RA-CD27-) and were highly cytotoxic against autologous tumor (89–100% Specific Lysis). Even a 2-day ex vivo activation generated highly cytotoxic T cells. The effectors were mostly CD4+ cells (62–90%). Majority of CD8+ T cells were central memory (CD45RA-/CD27-) or effector memory (CD45RA+/CD27-); a majority co-expressed intracellular granzyme B and perforin, 20–60% expressed PD-1. Most CD4+ co-expressed granzyme B and perforin, suggesting cytotoxic CD4+ T cells and were PD-1+. The in vitro cytotoxicity of expanded pleural T cells was highly reproducible among patients of significantly varied age (37–94) as well as patients with different molecular subtypes (triple negative, ER+PR+/Her2-, ER+PR-/Her2+, ER+PR+/Her2+, ER+AR+/PR-Her2-). Supernatants from cytotoxicity cultures evidenced secretion of G-CSF, IL-6, CXCL10, IFNγ, MCP-3, MIP1alpha and beta, IL-13, IL-2 and TNFalpha in a dose dependent fashion. Polyfunctional T cells (single cells secreting IL-2, TNFalpha, IFNγ, but not IL-10) were detectable among ex vivo expanded T cells.

Conclusions
Pleural T cells are not exhausted and can be stimulated to become potent anti-tumor effectors that may be useful for adoptive cellular therapy.

REFERENCES

Ethics Approval
Some samples were anonymized by an honest broker while some participants gave consent.