An autologous humanized patient-derived-xenograft platform to evaluate immunotherapy in ovarian cancer


Objective: The aim of this study was to “humanize” ovarian cancer patient-derived xenograft (PDX) models by autologous transfer of patient-matched tumor infiltrating lymphocytes (TILs) to evaluate immunotherapies.

Methods: Orthotopic high-grade serous ovarian cancer (HGSOC) PDX models were established from three patient donors. Models were molecularly and histologically validated by immunohistochemistry. TILs were expanded from donor tumors using a rapid expansion protocol. Ex vivo TIL and tumor co-cultures were performed to validate TIL reactivity against patient-matched autologous tumor cells. Expression of TIL activation markers and cytokine secretion was quantitated by flow cytometry and ELISA. As proof of concept, the efficacy of anti-PD-1 monotherapy was tested in autologous TIL/tumor HGSOC PDX models.

Results: Evaluation of T-cell activation in autologous TIL/tumor co-cultures resulted in an increase in HLA-dependent IFN-γ production and T-cell activation. In response to increased IFN-γ production, tumor cell expression of PD-L1 was increased. Addition of anti-PD-1 antibody to TIL/tumor co-cultures increased autologous tumor lysis in a CCNE1 amplified model. Orthotopic HGSOC PDX models from parallel patient-matched tumors maintained their original morphology and molecular marker profile. Autologous tumor-reactive TIL administration in patient-matched PDX models resulted in reduced tumor burden and increased survival, in groups that also received anti-PD-1 therapy.

Conclusions: This study validates a novel, clinically relevant model system for in vivo testing of immunomodulating therapeutic strategies for ovarian cancer, and provides a unique platform for assessing patient-specific T-cell response to immunotherapy.

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1. Introduction

Ovarian cancer is the fifth leading cause of cancer related deaths among women, resulting in nearly 14,000 deaths in the U.S. annually, with the majority of cases demonstrating high-grade serous histology [1]. Despite high response rates to aggressive surgical resection and first-line carboplatin–taxane chemotherapy, recurrence occurs in nearly 80% of cases. Patients with recurrent high-grade serous ovarian cancer (HGSOC) ultimately acquire platinum-resistance and succumb from disease [2,3]. For approximately 50% of HGSOCs, defects in homologous recombination (HR; e.g. BRCA1/2) cause sensitivity to PARP inhibitors (PARPi) [4,5], but complete response rates are low (2–9%) with partial responses being more common in the recurrent setting (35%) [6,7]. The other 50% of HGSOCs are HR-proficient, and frequently exhibit increased Cyclin E expression (~40%) either by CCNE1 gene amplification (20%) or CCNE1 copy-number gain (34%). Tumors with CCNE1 amplification are associated with poor overall survival and platinum-resistance [8–11]. Despite recent advances exploiting the genetics of ovarian cancer, most recurrent HGSOC ultimately develops drug resistance. Accordingly, alternative therapeutic strategies, we and others have applied patient-derived xenograft (PDX) models to facilitate optimization of immunotherapies in HGSOC. In order to perform clinical trials, have shown that PDX models reliably reproduce clinical outcomes [27,28]. However, these models of engrafted human tumors generally rely on immunodeficient mice that lack a human immune system, making it difficult to test immunomodulating therapies in vivo. To overcome this obstacle, humanization of xenograft and PDX models using healthy donor peripheral blood mononuclear cells (PBMCs) and hematopoietic stem cells (HSCs) have been created [29], however, these models lack an autologous adaptive immune response that has been primed against patient-specific tumor antigens.

We have now developed a “humanized” orthotopic PDX platform for HGSOC by performing autologous tumor-reactive TIL transfer, using T cells derived from the same patient’s tumor, in newly established orthotopic PDX models to create a platform for assessing patient-specific T-cell responses to immune therapy in vivo. We validated the utility of this novel preclinical patient-matched TIL/PDX model by testing both the impact of endogenous T-cell immunity and immune enhancing therapies, such as PD-1 inhibitors, on HGSOC progression. Our results demonstrate proof of concept for the humanized orthotopic HGSOC PDX platform as a valuable preclinical in vivo model system of host T-cell/tumor interaction that allows for assessment of patient-specific endogenous T-cell function, and for the testing of immune modulating agents and combination strategies in vivo.

2. Methods and materials

2.1. DNA sequencing and mutational analysis of patient tumors

Somatic mutation screening and copy number alteration analysis was performed on original patient tumors and PDX models using a custom designed targeted massively parallel sequencing protocol as previously described [24].

2.2. Autologous TIL-PDX models

Patient tumors were acquired from the Hospital of the University of Pennsylvania in accordance with IRB (#702679). NOD-SCID IL2RY–/– (NSG) mice were purchased from the Stem Cell Xenograft Core at the University of Pennsylvania (UPENN) and housed in accordance to UPPEN IACUC protocols (#806002). PDX models were generated as previously described [24]. In short, about three to three mm3 tumor chunks were sutured onto the fallopian tube/ovaries of 5 to 8-week-old female NSG mice, and 5–10 μl of Matrigel was placed over the transplant. When the tumor reached approximately 700–1,000 mm3, the tumor was excised and small tumor chunks again transplanted in a similar fashion for expansion (MP2). MP2 tumor was then used for preclinical studies. For all pre-clinical studies, mice were monitored daily, weights and condition scores were collected weekly, and tumor volume was measured by ultrasonod weekly. For WO-12 and WO-19 studies, when the tumor grew to 70–100 mm3, mice were randomized to treatment groups. Mice were injected with 1 × 107 autologous TILs i.p. weekly, and the following day with 10 mg/kg α-PD-1 or PBS (vehicle) i.p. This regimen was repeated for up to six weeks. For the WO-6 study, tumor was transplanted then 4 days later, 1 × 107 were TILs were injected followed by bi-weekly 10 mg/kg α-PD-1 or IgG4 i.p. administration. When the tumor volume reached >1000 mm3, mice were euthanized according to IACUC guidelines.

2.3. Histological analysis

Upon euthanasia, tumors were harvested and fixed in 10% neutral buffered formalin. Tissues were dehydrated in graded ethanol solutions, cleared in xylene, then embedded in paraffin. Blocks were cut into 5 μm sections and stained using the DAKO CoverStainer for H&E (Agilent, Santa Clara, CA).
Immunohistochemistry was performed using the DAKO EnVision Plus System with the DAKO Autostainer Plus Immunostainer, or the Leica Bond-III™ with the Bond Polymer Refine Detection System. Then the tissue was dehydrated and antigen retrieval was optimized using sodium citrate, pH 6.0 or EDTA, pH 9.0. Primary antibodies used for this study included CD3, p53, WT1 (DAKO), CD8 (Invitrogen), CD4 (Abcam), PD-L1, PD-1 (Cell Signaling), Pax8 (Cell Marque), and AE1/3 (Leica). Images were taken using a Leica DM 2000 microscope. For quantification of CD3⁺ T-cell infiltration, the average number of CD3⁺ T-cells per mouse was calculated from 40 to 60 10x fields.

2.7. TIL mediated cytolysis

To determine tumor lysis by patient-matched autologous TILs, co-cultures were analyzed on the xCELLigence Real-Time Cell Analysis (RTCA) multiplex system (ACEA Biosciences, San Diego, CA). Dissociated WO-19 PDX tumor cells or WO-12 primary cells were plated (ACEA Biosciences). Cell growth was dynamically monitored using RT-CES system (ACEA Biosciences) for 24–48 h. TILs at a 1:1 tumor to target ratio with or without anti-PD-1 were added (10 µg/mL) to tumor cell cultures.

2.8. Statistical analysis

When appropriate, results were reported as mean ± SD or mean ± SEM. Data were analyzed using one-way ANOVA with Dunnett’s multiple comparisons post-hoc test, or two-way ANOVA with Tukey’s multiple comparisons post-hoc test, or unpaired Students T-test. Additionally, in vivo survival studies were analyzed by Log-rank test and tumor volume over time was analyzed by area under the curve followed by a one-way ANOVA. Statistical significance was set at *<0.05, **<0.01, ***<0.001, ****<0.0001 (GraphPad Prism, La Jolla, CA). In vitro experiments were replicated at least three times.

3. Results

3.1. Development of PDX models for autologous humanization

PDX models recapitulate the characteristics of a patient’s original tumor, but lack the immune component known to control HGSOC progression [24,26,32,33]. We therefore sought to humanize PDX models by transplanting immune-deficient mice with patient ovarian tumors and infusing matched autologous-expanded tumor infiltrating lymphocytes (TILs), which would enable the evaluation of host immunity on cancer progression and the impact of immunotherapy (Fig. 1). Fresh ovarian tumor was transplanted to the ovaries of mice generating orthotopic PDX models as previously described [24]. In parallel, fresh tumor collected at the time of surgery was enzymatically dissociated and banked to establish primary tumor cultures for in vitro assays. TILs were isolated and expanded for in vitro co-culture assays and in vivo administration.

PDX tumor models and ex vivo TIL cultures were developed from three patient donors. The clinical and next generation sequencing data from the patient’s tumor are outlined in Table 1. Histologies were confirmed by a gynecologic pathologist. Patient tumor WO-12 and WO-6 were platinum-sensitive, whereas patient tumor WO-19 was platinum-resistant. The WO-19 tumor exhibited a CCNE1 amplification [9,10,34]. The patients’ original tumor morphology and expression of HGSOC markers, including paired box gene 8 (Pax8), p53, cytokeratin AE1/3, and Wilms’ Tumor 1 (WT1), was well maintained in PDX tumor models (Fig. 51). Ex vivo TIL cultures were also established for a previously developed and characterized PDX model, WO-6 [24]. This model is BRCA1 mutant (BRCA1mut) and was sensitive to platinum therapy. Patient tumors were tested for DNA mismatch repair proteins: MLH1, MSH2, MSH6, and PMS2. Expression was retained in all tumors, suggesting intact mismatch repair mechanisms.

3.2. Variation of T-cell populations and PD-1/PL-L1 axis in PDX models

One main advantage of PDX models is that they retain the tumor heterogeneity and to some degree, the stromal and immune compartment of the original tumor [24,26,32,33]. To evaluate the PDX immune microenvironment, PDX and original patient tumors were characterized for T-cell infiltration, as well as expression of
PD-1 and PD-L1 (Fig. 2A and B). WO-12 patient and PDX tumors had low expression of PD-1 (Fig. 2B), and were negative for PD-L1 by Immunohistochemistry (not shown). Human T-cell analysis determined that both patient tumors WO-12 and WO-19, had substantial infiltration of CD4+ and CD8+ T-cell populations, with an increased ratio of CD4+ cells. After two mouse passages, the WO-12 PDX model was virtually void of originating T-cells. In contrast, WO-19 tumor model retained CD4+ T-cells after two serial mouse passages, whereas all other models were lacking all CD4 and CD8 T-cells (Fig. S2). This suggests that some early passage orthotopic ovarian PDX models can partially maintain the compartments of the original patient immune-microenvironment initially but is significantly reduced after multiple passages.

3.3. Expansion and subset characterization of TILs from human HGSOC tumors

In order to humanize the orthotopic HGSOC PDX model, autologous patient-matched TILs were isolated and expanded for future administration. The methodologies applied for TIL isolation from patient tumor homogenate and expansion are outlined in Fig. 3A. Enzymatically digested tumors were initially cultured with Interleukin (IL)-7 and IL-15 to maintain and initiate the expansion of CD3+ T-cells, including the preferential expansion of the tumor-reactive TIL subset [31]. Growth rate was measured throughout the culture, and after 12–14 days in culture, 8- to 10-fold expansion of TILs was achieved (Fig. 3B and F). WO-12 TIL cultures consisted of 90% CD3+ cells with 71.5% CD8+CD3+ T-cells, and WO-19 TIL cultures had nearly 100% T-cell purity with 93.4% being CD8+CD3+ T-cells (Fig. 3B and F). In summary, expansion resulted in enrichment of the adaptive immune system, primarily cytotoxic and helper T cells to assess patient-specific T-cell responses.

3.4. TILs are reactive against patient-matched tumor cells

To assess whether expanded TILs were reactive against patient-matched tumor cells, ex vivo co-culture assays were performed, followed by analyses of T-cell cytokine secretion and expression of cell surface activation markers. WO-12 TILs co-cultured with WO-12 primary tumor cells resulted in increased frequencies of IFN-γ+ T-cells by flow cytometry based IFN-γ-capture assay, indicating T-cell activation (Fig. 3C). Pan-blockade of human leukocyte antigen (HLA) using MHC class I antibodies did not significantly reduce T-cell activation, suggesting a role for CD4+ cells in the response against autologous tumor cells. Here, the addition of PMA/I (Phorbol-Myristate-Acitate and Ionomycin) is a positive control for T-cell activation and IFN-γ secretion. Co-culture also resulted in increased frequencies of T-cells expressing the activation markers CD69 and IFN-γ+ T-cells (Fig. 3G, p < 0.0001). Similar to WO-12, WO-19 co-culture resulted in an increase in the frequency of T-cells expressing CD69 and PD-1 (Fig. 3H). Blocking with MHC class I neutralizing antibodies resulted in reduction of both IFN-γ and activation markers, signifying that activation of TILs is dependant upon interaction with peptide/HLA complexes, as shown previously [13].
The interaction of T-cell surface PD-1 with PD-L1 on tumor cells results in inhibition of T-cell signaling, cytokine production and proliferation [16,17]. IFNγ can also upregulate PD-L1 expression on tumor cells, thereby dampening effector T-cell surveillance by adaptive resistance. Flow cytometry was performed to evaluate PD-L1 expression by patient tumor cells before and after exposure to tumor-reactive TILs. PD-L1 expression on CD45+/CD45− tumor cells was low at baseline but increased for both donors when cultured with their respective patient-matched TILs (Fig. 3E and I), thus providing rational for assessing the impact of PD-1 checkpoint blockade on the cytolytic function of autologous TILs.

3.5. Anti-PD-1 increases cytotoxicity of autologous TILs against patient-matched tumor cells

To determine if autologous TILs have cytolytic activity against patient-matched tumor cells, co-cultures were analyzed for tumor lysis in real time using xCELLigence technology. Tumor cells were plated and TILs were added to the culture the following day. Tumor cell lysis was longitudinally monitored for up to 4 days. WO-12 TILs modestly lysed autologous tumor cells. (Fig. 4, left). By comparison, WO-19 TILs displayed robust tumoricidal activity against autologous tumor cells (Fig. 4, right; average area under the curve (AUC), \( p < 0.0001 \)). To determine whether blockade of the PD-1/PD-L1 axis enhanced TIL-mediated lysis, a PD-1 blocking antibody, pembrolizumab, was added to co-cultures. PD-1 blockade significantly increased the lytic activity of WO-19 TILs against autologous tumor cells (Fig. 4, right; AUC, \( p < 0.0001 \)), but had no detectable impact on WO-12 TIL-mediated lysis (Fig. 4, left). Together, these results suggest that WO-19 TILs may possess augmented antitumor activity in vivo, compared to WO-12 TILs. Also, the activity of WO-19 TILs may be enhanced further through co-administration of anti-PD-1 therapy, while WO-12 TILs would be unaffected by PD-1 blockade when applied in vivo.
Fig. 3. Expanded autologous TILs co-cultured with patient-matched tumor cells. (A) Schematic of TIL digestion and expansion. Patient tumors are first enzymatically and mechanically digested. Tumor digests are incubated with IL-7 and IL-15 for 48 h prior to pan T-cell isolation. T-cells were isolated and then cultured with irradiated artificial antigen presenting cells (aAPCs) that were pre-coated with anti-CD3 and/or anti-CD28 (Day 0). Starting on day 2, cultures were supplemented with 600 IU/ml of IL-2 and maintained at a cell concentration of 0.6–1 x 10^6 cells/ml. After 12–14 days in culture, expanded T-cells were assessed for their reactivity. TIL doubling throughout rapid expansion protocol and scatter plots for CD3+CD8+ characterization at day 14 shown for primary patient samples WO-12 (B) and WO-19 (F). Autologous TILs were co-cultured with patient-matched WO-12 (C–E) or WO-19 (G–I) tumor cells with or without addition of α-HLA blocking peptide or isotype control for 24 h. (C) WO-12 T-cell specific IFNγ secretion was analyzed with a flow cytometry-based assay. CD3+IFNγ+ T-cells were gated first by forward side scatter then live dead staining. WO-12 (D) and WO-19 (H) T-cell activation marker CD69 and inhibitory marker PD-1 were analyzed via flow cytometry. Cells were first gated on forward side scatter for lymphocytes and then for live cells using a live dead fixable stain. Results are reported for percent of CD45+CD3+ T-cells expressing CD69 or PD-1. WO12 (E) and WO-19 (I) Tumor cell staining of T-cell inhibitory marker PD-L1 was analyzed via flow cytometry. Live tumor cells were gated first by forward side scatter then live dead staining. CD45+EPCAM+PD-L1+ cells were analyzed for mean fluorescence intensity (MFI) of PD-L1. MFI of isotype control was subtracted from MFI of CD45+EPCAM+PD-L1+ cells alone or co-cultured with TILs. (G) WO-19 co-culture supernatant was analyzed for IFNγ secretion by ELISA and reported as concentration (pg/mL). Data reported is a representative of three individual experiments and was analyzed by one-way ANOVA and Tukey’s multiple comparison post-hoc test or unpaired T-test when appropriate. Mean ± SD (n = 3), *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
3.6. Autologous TILs in combination with anti-PD-1 in an orthotopic HGSOC PDX model

Clinically relevant HGSOC models that can be utilized to test the in vivo efficacy of immune therapies, such as checkpoint inhibitors, are lacking. With the co-development of well-characterized orthotopic HGSOC PDX models and patient-matched, tumor-reactive TIL cultures, we sought to develop a humanized PDX model with two goals. First, we sought to test the impact of the patient’s immune system on tumor progression in vivo. Second, we aimed to enhance the endogenous immune reactivity through the application of immune checkpoint inhibition. NSG mice were orthotopically transplanted with MP3 tumors for pre-clinical studies (Fig. 5A). When PDX tumors reached 70–100 mm³, mice were randomized into TIL infusion alone, TIL infusion + anti-PD-1, or vehicle control groups. Upon randomization, 1 x 10⁷ autologous TILs were administered by intra-peritoneal (i.p.) injection weekly. Following TIL injections, mice received 10 mg/kg anti PD-1 by i.p. injection, twice per week. This dosing strategy was repeated over a 6 week course, or until the mouse was euthanized due to tumor volume exceeding 1000 mm³.

In the WO-12 PDX model, administration of TILs significantly reduced tumor volume at 6 weeks (Fig. 5B, 2-way ANOVA, p = 0.0098), but did not significantly impact survival compared to the vehicle control group (Log-rank, p = 0.3430). The median survival of untreated mice was 8 weeks, compared to a median survival of 18 weeks for mice that received TIL transfer, however due to small sample size these effects did not reach significance (Log rank test, p = 0.2122). In this model, the addition of anti-PD-1 antibody had no significant additive effects on CD3⁺ T-cell infiltration, tumor volume or survival (Fig. 5B and S3A), mimicking the results of coculture assays (Fig. 4).

In comparison, the studies utilizing the WO-19 TIL/PDX model did result in significant effects when anti-PD-1 therapy was administered. At four weeks post-treatment, groups receiving autologous TILs alone had modestly reduced tumor volumes compared to vehicle control mice (AUC, p = 0.5349; 2-way ANOVA, p = 0.0053). Mice administered both TIL and anti-PD-1 has significantly reduced average tumor volume compared to untreated WO-19 mice (AUC, p = 0.0202; 2-way ANOVA p < 0.0001, Figs. 5C and S3B). TILs and anti-PD-1 significantly reduced tumor volume compared to TILs alone at five and six weeks post-randomization (2-way ANOVA, p = 0.0317 and p = 0.0002, respectfully). TIL infusion alone did not increase overall survival compared to untreated mice, however, TIL infusion plus anti-PD-1 therapy increased overall survival (Log rank test p = 0.0057) compared to the TILs alone and the vehicle control groups (p = 0.0084 and p = 0.0069, respectfully). For mice humanized with TILs, the addition of anti-PD-1 therapy increased the median survival from 5 to 7 weeks, compared to untreated mice (ANOVA, p = 0.0022, Fig. 5C). Five weeks post-randomization, all of the mice in the untreated groups had tumors with volumes >1000mm³ and were euthanized, whereas all mice who received autologous TILs plus anti-PD-1 had lower tumor burden (Fig. 5C).

The primary goal of checkpoint inhibitor treatment is to bolster endogenous anti-tumor T-cell activity. Upon euthanasia, tumors were collected and IHC was performed in order to assess relative intraepithelial CD3⁺ T-cell accumulation amongst the various treatment groups. Representative images of CD3⁺ staining are shown (Fig. 5D). Although the overall number of infiltrating T-cells was not significantly different between treatments groups, there was a trend toward an increase in the average number of intraepithelial T-cells in the TIL plus anti-PD-1 group (Fishes Least Squared Difference Test p = 0.0390), which correlated to overall survival and tumor burden.

Clear cell carcinomas are generally less responsive to chemotherapy [35,36], but may have increased sensitivity to immunotherapy [37]. We therefore utilized an established PDX model derived from a mixed HGSOC with clear cell to test the activity of anti-PD-1 antibody therapy. Prior to in vivo studies, we tested tumor reactivity in ex vivo co-cultures and found that similar to WO-19, IFNy secretion and CD69 surface expression is upregulated in an HLA 1 dependent manner. MHC II neutralizing peptides did not further reduce the level of CD3 activation, suggesting that CD8⁺ T-cells, not CD4⁺ T-cells, are the major mediators of the response to autologous tumor cells in this model (Fig. S5C).

To determine if anti-PD-1 therapy was efficacious at reducing tumor burden in the WO-6 TIL/PDX model, an endpoint in vivo study was performed. Since the WO-6 PDX model showed increased baseline growth kinetics, tumors were orthotopically transplanted, and then mice were randomized and administered with 1 x 10⁷ autologous TILs four days later (Fig. S4D). One and four days following each TIL administration, mice received 10 mg/kg anti PD-1 or IgG antibody by i.p. administration. Most untreated mice required euthanization due to tumor progression (>1000 mm³ tumor volume) by 20 days. Upon euthanization at day 20, CD3⁺ and CD3⁺CD8⁺ peripheral T cells were quantified by flow cytometry. The addition of IgG4 to TIL administration did not significantly increase the number of peripheral CD3⁺ or CD3⁺CD8⁺ T cells compared to the TILs alone arm. However, administration of anti-PD-1 antibody did significantly increase the quantity of circulating T-cells compared to TILs alone (CD3⁺, p = 0.0289; CD3⁺ CD8, p = 0.0258, Fig. S4E). Mice treated with TILs and anti-PD-1 showed a significant reduction in tumor burden, compared to TILs
with IgG4 isotype control (ANOVA, $p = 0.003$, Fig. S4F). There was a trend for an increased number of intraepithelial T-cells (CD3\(^+\)) in all groups that were administered TILs, however this only reached statistical significance for the group treated with TILs alone (ANOVA, $p = 0.0457$, Fig. S4G). In summary, we demonstrate that the autologous patient-matched TIL/PDX model is a unique platform to assess patient-specific T-cell response to immunotherapy.

4. Discussion

Advanced stage HGSOC often recurs and cure is relatively rare [38]. Immunotherapies have significantly improved patient outcome in several non-gynaecological cancers such as melanoma, lung, renal and colon cancer leading to FDA approval [18–20]. However, immunotherapies to date have demonstrated only
modest clinical responses for HGSOC [20,21]. Immunomodulatory agents as monotherapies will likely be inadequate in the recurrent setting for this disease [20,23]. To date, we have not optimized when (e.g. upfront treatment, maintenance setting, or at time of recurrence) and how (e.g. in combination with cytotoxic drugs or with other immunotherapies, or by concomitant vs sequencing schedules) immunotherapies should be utilized in HGSOC. Preclinical model systems that mimic the biology of the original patient tumor and incorporate the patient’s immune system are critical to understand, and optimize immunotherapies to ultimately guide human clinical trial design.

There are a lack of preclinical in vivo models that both accurately recapitulate human HGSOC and enable the testing of immune modulating therapies that effect adaptive immune responses. Current model systems generally utilize immunocompetent syngeneic and genetically engineered mice, or immune-deficient HGSOC xenograft mouse models. Of these models, only xenograft models bear human tumors, and since these models generally lack a functional immune system, they are ineffective for testing immunotherapies. To overcome this challenge, three general models have been developed which harbor a limited immune component reconstituted in vivo [29]. First, models have been humanized by inoculating healthy human PBMCs from donors, which contain mature human T-cells. Donor PBMCs are readily available and cost effective, but are often immunologically mismatched to the tumor and rarely possess autologous anti-tumor activity. Second, models have been humanized by inoculating human CD34+ cells, stem cells derived from the bone marrow, umbilical cord, or fetal thymus. In this case, all human hematopoietic lineages are represented, but not all are functionally developed. T-cells developing in this system seldom experience central tolerance induction, have not been previously exposed to the hostile tumor microenvironment, and are seldom matched to the engrafted tumor. Also, erroneously trained T-cells and other cells from the hematopoietic system frequently cause graft-vs-host disease [28]. Although these preclinical models can be informative, they lack a tumor-primed, autologous immune component that may better predict patient-specific responses in a heterogeneous disease such as HGSOC.

Here we developed a two-tiered platform for testing the efficacy of novel immunomodulating therapeutics or combinations, in autologous patient-matched TIL/PDX models. First, TIL reactivity was tested ex vivo in order to provide an indication of the predictive response to therapeutics in vivo. Expanded TILs were activated when co-cultured with patient-matched tumor cells in vitro, signified by an HLA-dependent increase in IFNγ production and expression of cell surface activation markers CD69 and PD-1. Moreover, tumor-specific expression of PD-L1 was induced in the presence of autologous TILs. Due to the increased expression of PD-L1 on T-cells and concomitant PD-L1 on tumor cells, studies were conducted to determine if disruption of the PD-1/PD-L1 axis would increase tumor reactivity of the expanded TILs. Anti-PD-1 was added to TIL and tumor co-cultures and tumor lysis was analyzed. These studies demonstrated that the addition of anti-PD-1 therapy in one model (WO-19) increased tumor lysis ex vivo, which rationalized the testing of anti-PD-1 therapy in vivo.

The second tier of the platform utilized a novel orthotopic HGSOC PDX model transferred with patient-matched tumor-reactive TILs to test the efficacy of anti-PD-1 checkpoint inhibitor therapy. These PDX models maintain the genetic characteristics of the primary patient tumor however much of the immune cell components do not persist after serial passaging. Immunohistochemical profiling of the T-cells indicated that some human CD3 T-cells are maintained in the WO-19 PDX tumors however almost all human CD8+ T-cells are diminished. We find that reintroduction of CD8+ TILs controlled HGSOC progression in vivo, which could be enhanced by the addition of immune checkpoint blockade therapy (Fig. 5 and S4).

Anti-PD-1 therapy was tested in the three distinct and newly developed autologous TIL/PDX models of HGSOC. In the WO-12 model, the addition of anti-PD-1 did not increase survival in mice. These results were not surprising since ex vivo co-culture results indicated reduced CD8+ TIL activity (Fig. 3) and a minimal impact from the addition of anti-PD-1 to the tumor lysis assay (Fig. 4). Moreover, the WO-12 PDX model displayed lower overall PD-1 staining and fewer CD4 T-cells retained from the original patient tumor (Fig. 2) when compared to the WO-19 model. However, in the WO-19 (CCNE1 amplified) model, TIL infusion plus anti-PD-1 reduced overall tumor burden, and significantly increased survival compared to TIL alone and vehicle treated mice. Compared to WO-12, PDX models from this donor had higher PD-1 expression in regions with residual CD4+ T-cells, which could have an added effect when treated with anti-PD-1. In a highly aggressive WO-6 model of BRCA1mut HGSOC with clear cell changes, tumor burden was significantly reduced when TILs and anti-PD-1 were administered, compared to when TILs were administered without the IgG control. This finding is of particular interest since many clear cell ovarian carcinomas are characterized by an immunosuppressive microenvironment [39], high microsatellite instability or mismatch repair deficiency, increased neoantigen burden [40], and PD-L1 expression by tumors [41] and the expression of PD-1 on TILs and PD-L1 on tumor cells is reported to correlate with clinical outcome [42,43]. More so, patients with clear cell ovarian carcinomas may be more sensitive to immune checkpoint blockade therapy using PD-1 and PD-L1 inhibitors [22,44,45] than other ovarian cancer histologies. As such, the WO-6 model TIL/PDX model provides a unique opportunity to identify and enhance the mechanisms controlling responsiveness to immunotherapy in this subset of ovarian cancer patients.

In the examination of tumors resected at the end of study, treatment with TILs did not appear to induce sustained PD-L1 expression in the tumor microenvironment in the TIL/PDX models (data not shown). It remains possible that, in the early aftermath of TIL infusion, locally secreted IFNγ by transferred TILs results in transient increases in tumor PD-L1 expression, thereby creating a PD-1/PD-L1 axis of immune suppression which was overcome by the administration of anti-PD-1 therapy. Deeper longitudinal studies will aid in understanding the underpinnings of anti-PD-1 therapies in this model, however, it may be expected that greater efficacy may be achieved in additional TIL/PDX models that have higher baseline endogenous PD-L1 expression in vivo.

Our new humanized TIL/PDX platform has been shown to have preclinical applications when testing immunotherapies in vitro and in vivo. Unlike other preclinical models, our humanized TIL/PDX models utilize patient-matched TILs to evaluate the anti-tumor effect of immunomodulating therapies against autologous-tumors. As these TILs have already been primed to the heterogeneity of the autologous tumor, ex vivo engineering of the T-cell receptor repertoire is unnecessary. However, there are still some inherent limitations to the current model, for example, a lack of innate immune cells (i.e. tumor associated macrophages), and the known chimeric nature of the tumor vasculature. Furthermore, the development of each model remains reliant on the availability of patient tumor material and successful TIL expansion. Yet, the autologous nature of this system is designed to limit allogeneic immune responses against cancer and to serve as a platform for the testing of highly personalized, patient-specific responses to immune interventions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gyno onc.2019.10.011.

Declaration of competing Interest

The authors declare no potential conflicts of interest.

Author contribution

S.B.G., H.K., S.R., A.G.R. participated in experimental design, conducted experiments, collected, analyzed and interpreted data. D.O. processed primary tumor samples, performed in vitro cell expansion, and performed blood analysis. S.M., Y.K. assisted with in vivo studies. A.J.F. assisted with immunohistochemistry. H.X. established and provided primary tumor cultures. L.E.S. compiled and analyzed data. D.J.P. participated in experimental design, contributed to data analysis and interpretation. F.S. provided primary patient samples, developed the orthotopic transplant PDX models, participated in experimental design, contributed to data analysis and interpretation. S.B.G. wrote the manuscript. All authors assisted in writing and editing the manuscript.

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