Genomic and functional fidelity of small cell lung cancer patient-derived xenografts

Benjamin J. Drapkin1*, Julie George2*, Camilla L. Christensen3, Mari Mino-Kenudson4, Ruben Dries5, Tilak Sundaresan1,5†, Sarah Phat1, David T. Myers1, Jun Zhong1, Peter Igo1‡, Mehlika H. Hazar-Rethinam1, Joseph A. Licausi1,5, Maria Gomez-Caraballo1††, Marina Kem1,4, Kandarp N. Jani3, Roxana Azimi1, Nima Abedpour2,6, Roopika Menon7, Sotirios Lakis7, Rebecca S. Heist1,5,13, Reinhard Büttner8, Stefan Haas9, Lecia V. Sequist1,5,13, Alice T. Shaw1,5,13, Kwok-Kin Wong16, Aaron N. Hata1,5,13, Mehmet Toner5,10,11,12, Shyamala Maheswaran1,5,11, Daniel A. Haber1,5,13,14, Martin Peifer2,6, Nicholas Dyson1,5, Roman K. Thomas2,8,15, Anna F. Farago1,5,13

1 Massachusetts General Hospital Cancer Center, Boston MA
2 Department of Translational Genomics, Center of Integrated Oncology Cologne–Bonn, Medical Faculty, University of Cologne, 50931 Cologne, Germany.
3 Dana-Farber Cancer Institute, Boston MA
4 Department of Pathology, Massachusetts General Hospital, Boston MA
5 Harvard Medical School, Boston MA
6 Center for Molecular Medicine Cologne (CMMC), University of Cologne, 50931 Cologne, Germany
7 NEO New Oncology GmbH, Gottfried-Hagen-Str. 20, 51105 Cologne, Germany
8 Department of Pathology, University Hospital Cologne, 50937 Cologne, Germany
9 Computational Molecular Biology Group, Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany
10 Center for Engineering in Medicine, Massachusetts General Hospital, Boston MA
11 Department of Surgery, Massachusetts General Hospital, Boston MA
12 Shriners Hospital for Children, Boston MA
13 Department of Medicine, Massachusetts General Hospital, Boston MA
14 Howard Hughes Medical Institute
15 German Cancer Research Center, German Cancer Consortium (DKTK), Heidelberg, Germany
16 Department of Hematology and Oncology, New York University Langone Medical School, New York NY

* Co-first authors
† Current address: Kaiser Permanente, San Francisco, CA
‡ Current address: Sentien Biotechnologies, Lexington, MA
†† Current address: Duke University Medical School, Durham, NC

Running Title: Genomic and functional fidelity of SCLC PDX models

Keywords: small cell lung cancer, patient-derived xenografts, circulating tumor cells

Additional Information:
- Financial Support:
This work was supported by the V foundation for Cancer Research (T2016-003 to ND and AFF), a grant from Uniting Against Lung Cancer: New England (UALC2014 to AFF), Conquer Cancer Foundation of ASCO Young Investigator Awards (YIA 2014 to AFF and YIA 2017 to BJD), the President and Fellows of Harvard College (CMerIT to AFF), the National Institute of Health and National Cancer Institutes (Chabner K12CA087723 to AFF, U24CA213274 to AFF, K99CA201618 to CLC, 1U01CA213333-01 to KKW, NCI 2RO1CA129933 to DAH; NIH Quantum 2U01EB012493 to MT and DAH), the Bridge Project – a collaboration between The Koch Institute for Integrative Cancer Research at MIT and the Dana-Farber/Harvard Cancer Center (DF/HCC) (to KKW), Howard Hughes Medical Institute (to DAH), National Foundation for Cancer Research (to DAH), the German Ministry of Science and Education (BMBF) as part of the e:Med program (grant no. 01ZX1303A to KKW, RB and MP, and grant no. 01ZX1406 to MP), by the Deutsche Forschungsgemeinschaft (DFG; through TH1386/3-1 to KKW and CRU-286 to MP), by the Deutsche Krebshilfe as part of the Oncology Centers of Excellence funding program (to KKW), by the German Cancer Consortium (DKTK) Joint Funding program, and by the International Association for the Study of Lung Cancer (IASLC Young Investigator Award to JG).

- **Corresponding authors:**
  Anna F. Farago, M.D., Ph.D.
  Massachusetts General Hospital Cancer Center
  55 Fruit Street, Yawkey 7B
  Boston MA 02114
  USA
  Phone: 617-643-3472
  Fax: 617-726-0453
  Email: afarago@partners.org

  Roman K. Thomas, Ph.D.
  Universität zu Köln
  Abteilung Translationale Genomik
  Weyertal 115 b
  50931 Köln
  Germany
  Phone: 49-221-478-98777
  Fax: 49-221-478-97902
  Email: roman.thomas@uni-koeln.de

- **Conflicts of interest:**
  AFF: Received consulting fees from AbbVie, Pharmamar SA. Received research funding (to institution) from AstraZeneca, AbbVie, Pharmamar SA, Bristol-Myers Squibb, Merck, Loxo Oncology, Ignyta Inc, Novartis.
RSH: Received consulting fees from Boehringer Ingelheim, Ariad. Research funding (to institution) from Novartis, Celgene, Genentech/Roche, Millenium, Debiopharm, Peregrine, Incite, CORvus, Mirati, Abbvie.
LVS: Received consulting fees from AstraZeneca, Bristol-Myers Squibb, Genentech, Pfizer. Unpaid consulting for Novartis, Boehringer Ingelheim, Merrimack.
MT, SM, DAH: Massachusetts General Hospital has filed for patent protection for the CTC isolation technology.
RB: Cofounder and owner of Targos Molecular Diagnostics and received honoraria for consulting and lecturing from AstraZeneca, Boehringer Ingelheim, Merck, Roche, Novartis, Lilly, and Pfizer.
RKT: Founder of NEO New Oncology GmbH, now part of Siemens Healthcare. Received consulting and lecture fees from Merck, Roche, Lilly, Boehringer Ingelheim, Astra-Zeneca, Daiichi-Sankyo, MSD, NEO New Oncology, Puma, Clovis.
BJD: research funding (to institution) from Novartis
ND: research funding (to institution) from Novartis
All other authors have no conflicts of interest to report.

**Manuscript information:**
Total word count (excluding references and figure legends): 7096
Total number of figures and tables in main text: 6
Total number of figures and tables in supplement: 19
ABSTRACT

Small cell lung cancer (SCLC) patient-derived xenografts (PDXs) can be generated from biopsies or circulating tumor cells (CTCs), though scarcity of tissue and low efficiency of tumor growth have previously limited these approaches. Applying an established clinical-translational pipeline for tissue collection and an automated microfluidic platform for CTC-enrichment, we generated 17 biopsy-derived PDXs and 17 CTC-derived PDXs in a two-year timeframe, at 89% and 38% efficiency, respectively. Whole exome sequencing showed that somatic alterations are stably maintained between patient tumors and PDXs. Early-passage PDXs maintain the genomic and transcriptional profiles of the founder PDX. In vivo treatment with etoposide and cisplatin (EP) in 30 PDX models demonstrated greater sensitivity in PDXs from EP naïve patients, and resistance to EP corresponded to increased expression of a MYC gene signature. Finally, serial CTC-derived PDXs generated from an individual patient at multiple time points accurately recapitulated the evolving drug sensitivities of that patient's disease. Collectively, this work highlights the translational potential of this strategy.
STATEMENT OF SIGNIFICANCE

Effective translational research utilizing SCLC PDX models requires both efficient generation of models from patients and fidelity of those models in representing patient tumor characteristics. We present approaches for efficient generation of PDXs from both biopsies and circulating tumor cells (CTCs), and demonstrate that these models capture the mutational landscape and functional features of the donor tumors.
INTRODUCTION

SCLC is a high-grade neuroendocrine malignancy with a 5-year overall survival of approximately 5%. Among patients diagnosed with metastatic (extensive stage) disease, the median overall survival is approximately 9-11 months with standard treatment [1-4]. Because neither surgical resection nor repeat tumor biopsies are standard of care in metastatic SCLC, access to clinically relevant tissue is limited. Instead, SCLC research has relied heavily on preclinical models such as established cell lines, genetically engineered mouse models (GEMMs), and more recently, PDXs [5-8].

PDX models, generated via direct implantation of patient tumor tissue into a recipient mouse, have several theoretical advantages over other preclinical models. First, bypass of an in vitro intermediate potentially avoids bottleneck events or selection for fitness in the in vitro environment [9]. Second, molecular and functional phenotypes can be correlated directly with patient characteristics and clinical response to therapies. The increasing use of PDX models in SCLC research underscores their importance to the field [5, 10, 11].

Many solid tumors shed malignant cells into circulation, and collection of these rare CTCs enables non-invasive serial tumor sampling [12-15]. CTCs are highly abundant in SCLC patients compared to patients with other solid tumor malignancies [16-18], and CTC number is prognostic and reflects the changing burden of disease over the course of treatments [19]. The development of PDX models from CTCs isolated by Ficoll density gradient marked a significant advance for generation of SCLC preclinical models [20], eliminating the need for surgical resection or invasive biopsy. To
date, a handful of CTC-derived SCLC PDX models have been described [11, 20]. These were generated from samples that had a minimum of 400 CTCs per 7.5 mL blood, but the widespread application of this methodology to generate SCLC models has yet to be reported. Although live CTC enrichment can be achieved through application of microfluidic devices [21], this approach has not yet been used for PDX generation.

While the application of SCLC PDXs for preclinical studies is increasingly common, further characterization is needed to assess how accurately these models reproduce properties of the human disease. SCLC tumors are known to have extensive genomic alterations and a high mutational burden [22-24]. Furthermore, bi-allelic inactivation of \( TP53 \) and \( RB1 \) is nearly universal in SCLCs [22]. These changes may undermine genomic stability, raising the concern that SCLC genomes may acquire additional genomic alterations and evolve rapidly when passaged in mice. The recent finding that PDX models of diverse tumor types acquire mutations with serial passages underscores this concern [25]. While copy number variations appear conserved between CTCs and CTC-derived PDXs in a limited number of cases examined [20], whether the genomes of CTC-derived PDX models of SCLC accurately mirror the patient’s biopsy has not yet been rigorously investigated.

Functional characterization of SCLC PDXs is also a critical metric of the utility of these models. SCLC tumors are classically highly sensitive to etoposide and platinum (EP) [1]. Among 3 PDX models treated with EP [20], responses in the models correlated with those in the donor patients. However, more extensive testing of how well SCLC PDXs recapitulate chemotherapy sensitivity and acquired resistance, and how these profiles correlate with underlying molecular signatures, is needed.
Here we describe the efficient generation of 34 PDX models of SCLC in a two-year time frame, utilizing both tissue biopsies and blood samples processed with an automated microfluidic device. We show that these models can be generated at high efficiency from CTCs, irrespective of enumeration, and that they faithfully recapitulate both the genomic and functional features of patient tumors at the time of model generation. We quantified in vivo sensitivity to EP in 30 PDX models, and found that models derived from EP naïve patients were more sensitive than models from patients previously treated with EP. Further transcriptome profiling of models revealed that increasing EP resistance correlates closely with activation of a MYC transcription program. Finally, serial models derived from an individual patient at multiple time points reflect the evolving clinical response of that patient’s tumor, emphasizing the potential application of these approaches toward studying acquired resistance.

RESULTS

Construction of a SCLC PDX panel

Biopsy- and CTC-derived PDX models from SCLC patients have been described [5, 20, 26] but there remains a paucity of SCLC models from patients with detailed correlative clinical data. PDXs generated after patient relapse are especially scarce because these patients rarely undergo a clinically indicated biopsy. Currently, there are no published sets of sequential PDX models from SCLC patients that can be used to study disease evolution. The key parameter, as yet uncertain, is the success rate of PDX generation from routine blood samples and tissue biopsies. We therefore focused
on a population of SCLC patients selected only on the basis of having clinically progressive disease at the time of tissue or blood collection.

Between June 2014 and June 2016, we enrolled patients with known or suspected SCLC on IRB-approved protocols for collection of clinical data, blood and tissue. Blood samples for PDX development were taken at the time of initial diagnosis or progression after a prior therapy, but not while a patient’s disease was currently responding to treatment. For each patient, SCLC CTCs were isolated from a 15-20 mL whole blood sample with the CTC-iChip\textsuperscript{neg} device [27], with a typical transport time from patient to laboratory of 60-90 minutes. The CTC-iChip\textsuperscript{neg} first excludes plasma, unbound antibody-coated beads, platelets and red blood cells through microfluidic size-based separation, and then directs nucleated cells (leukocytes and CTCs) into a single-cell stream through inertial focusing microfluidic channels, thereby enabling the highly efficient magnetic separation of anti-CD45/CD66b-tagged leukocytes [27, 28]. The resulting product is highly enriched (10\textsuperscript{4}-10\textsuperscript{5}-fold) for unmanipulated and potentially viable CTCs (Figure 1A). CTC-enriched products were immediately prepared for subcutaneous injection into NOD \textit{scid} gamma (NSG) mice. Animals were then monitored for tumor growth over a period of at least 6 months. From 42 processed blood samples, 16 animals developed palpable tumors, for an overall tumor growth efficiency of 38\% (95%CI 24-54) and a median latency of 115 days (Figure 1B-D). Following previously described protocols for [20], we generated 1 model from CTCs isolated by Ficoll gradient (Supplementary Table S1).

In a parallel effort, we also sought to generate PDX models of SCLC from patients undergoing a tissue biopsy. One extra core tumor biopsy was collected from
patients with known or suspected SCLC when they were undergoing a biopsy for clinical purposes. Core tumor specimens, generally 19-22 gauge and approximately 4-8 mm in length, were obtained from patients and implanted into the flanks of NSG mice within 2 hours of the biopsy procedure (Figure 1A and Supplementary Table S2). From 18 implanted specimens from patients with confirmed SCLC, 16 developed into xenograft tumors within 6 months, for an overall growth efficiency of 89% (95% CI 74-99) and a median latency of 78 days (Figure 1B, C and Supplementary Table S1). In addition, one PDX model was generated from a malignant pleural effusion.

Once the founder (P0) PDX tumors reached a size of approximately 1-1.5 cm in diameter, they were dissected, portions of the tumor material were passaged into additional NSG mice and tissue was preserved for further analyses (Figure 1A). The latency for growth of passaged tumors was typically 2-6 weeks, considerably shorter than for P0 tumor growth. There were no model failures after initial P0 tumor emergence; all xenografts were successfully maintained for at least 2 passages, and all attempts at regrowth from cryopreserved specimens were successful (Supplementary Table S1). In total, within the specified timeframe for tissue collection, 34 SCLC models were established from 27 separate patients (Figure 1B). These models were generated from patients with a range of time points in their clinical course of SCLC, including 15 models from patients prior to receiving any SCLC-directed therapy, and 19 from patients after at least one line of therapy. From three patients, serial models were developed at multiple points over the course of their treatments (Figure 1B).

To confirm that the PDX tumors were pathologically consistent with SCLC, histological and immunohistochemical analysis of the P0 PDX models was performed.
by a thoracic pathologist (MMK), and compared with the patient biopsy when available. In all cases examined, the PDX model demonstrated histologic and immunohistochemical features consistent with SCLC, including neuroendocrine marker expression and absence of nuclear Rb (representative examples are shown in Figure 1E and Supplementary Figure 1). Detection of CD45 was used to rule out lymphoproliferation, and was negative in all 30 models tested (Figure 1E, Supplementary Figure 1, and data not shown). Histologic comparison of PDX and corresponding patient biopsy samples showed strong similarity across the models (Supplementary Table S1).

Genomic characterization of SCLC PDX models

We selected 7 PDX models for whole exome sequencing (WES) (Table 1) and examined the somatic alterations in these models (Supplementary Table S3-S4). To account for contaminating mouse tissue DNA in the PDX tumor samples, computational analyses excluded mouse reference sequencing reads (Methods). Consistent with the known genomic hallmarks of SCLC [22-24, 29, 30], WES aided in the identification of inactivating alterations of TP53 and RB1 in all models and confirmed bi-allelic loss of these tumor suppressor genes in most cases (Figure 2A, Supplementary Table S4). MGH1514-1 and MGH1512-1 were only found with heterozygous loss of TP53 and RB1, respectively, which may be due to the technical limitations of WES in detecting larger complex genomic rearrangements that commonly affect these loci in SCLC. However, paired-end transcriptome sequencing of MGH1514-1 revealed an out-of-frame chimeric transcript harboring TP53 exon 1 fused to ITNL2 exon 8 (Figure 2B), thus implicating bi-
allelic genomic loss of TP53 in this tumor. While bi-allelic genomic alterations of RB1 were not detected in MGH1512-1, this tumor had low abundance of RB1 transcripts (Figure 2D) and lack of Rb staining in IHC (Figure 1E), indicating a functional loss of Rb1 in this tumor.

Additional alterations were found in genes encoding chromatin-modifying enzymes (e.g. CREBBP, EP300 and MLL3), SLIT2 and NOTCH1 (Figure 2A and Supplementary Table S4), which were previously identified as significant alterations in SCLC [22-24]. Although events that augment MAP kinase pathway activity are thought to be unusual in SCLC, two models harbored alterations in this pathway (Figure 2A): MGH1525-1 had a point mutation in the DFG motif of BRAF thought to activate MEK/ERK signaling (BRAF<sup>G596C</sup>) [31, 32]; and MGH 1514-1, which was derived from a never-smoker with de novo SCLC, harbored an activating EGFR mutation (EGFR<sup>del_exon19</sup>). Neither case had any histologic evidence of concurrent non-small cell lung cancer. All tumors with the exception of MGH1514-1 revealed a high rate of cytosine to adenine (C:A) nucleotide transversions (Figure 2A, Supplementary Table S3) which reflects tobacco induced mutagenesis [33] and which is consistent with the smoking history of the patients.

To complement the genomic study of the models, transcriptome sequencing was performed to determine the expression profiles of serial passages (P0 and P2) (Supplementary Table S5). In order to assess the fidelity of these expression profiles within a given model, we compared the transcripts with the highest variability across all samples to generate a correlation matrix (Figure 2C). Paired samples from the same model correlated tightly, and were uncorrelated with samples from other PDX models.
which emphasizes reproducible expression profiles among biological replicates from different passages of the same PDX. While paired-end transcriptome sequencing could not be performed on the matched patient biopsies, the PDX mRNA expression profiles were mapped to a previously published database of 20 human tumors [22]. Using the defining features of the dominant clusters within this dataset, 6/7 PDX models showed strong similarity with the neuroendocrine-high profile, with only one PDX, MGH1515-1, clustering with the neuroendocrine-low tumors (Figure 2D). Although the dominant PDX expression profiles mapped to primary tumor clusters, each model harbored distinct and patient-specific transcriptional signatures (Supplementary Figure 2D). To investigate these PDX-specific features, we identified transcripts that correlated strongly with either high expression in one model, or absent expression in one model, and then filtered through the Molecular Signatures Database (MSigDB V6.0) preset gene families lists for cancer-related genes (Supplementary Figure 2 and Supplementary Table S6). Interestingly, this analysis highlighted changes in the expression in pathways known to be important in SCLC. None of the models showed high-level amplification of any MYC family genes, but each MYC family member displayed elevated expression in one specific PDX model (Supplementary Figure 2). While the Notch signaling pathway has been implicated in the development of SCLC [22, 34-36], transcriptome sequencing revealed model-specific expression of NOTCH receptors and ligands. MGH1514-1, which harbors an activating EGFR mutation, is distinguished by elevated expression of EGFR as well as other MAP kinase pathway components including ARAF, CRAF and MEKK1 (Supplementary Figure 2 and Supplementary Figure 3). Notably, EGFR mutations have been described in rare cases of SCLC in never-smokers [37], and
SCLC transformation is an established mechanism of acquired resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer [38-41]. Thus, activated pathways and gene families vary across models, suggesting that the outcome of functional studies may greatly depend on the specific PDX model used for investigation.

**Genomic fidelity of PDX models compared to patient biopsies and over serial passages**

We conducted comparative genomic analyses on patient-derived biopsies versus PDX models to address three important topics: (i) the genomic fidelity of the PDX models compared to patient tumor specimens collected at the same time point, (ii) the question of whether CTC-derived models are more divergent from the primary tumor than biopsy-derived models, (iii) the question of whether genetic drift occurs during serial passaging of SCLC tumors in NSG mice.

To address the fidelity with which the xenograft models reflect primary tumors, we compared the exomes of CTC- or biopsy-derived PDX models with patient tumor biopsies collected from the patients at the same time point (Figure 3A). WES was performed on patient tumor biopsy tissue, xenograft tumors at passage 0 (P0), xenograft tumors at P1 or P2, and patient-matched germline DNA. Among the selected series, there were 4 sets of PDX tumors derived from CTCs and 2 sets derived from biopsies. For one of our sequenced PDX models, MGH1528-1, no corresponding patient tumor biopsy material was available (Table 1).
Comparison of copy number alterations between patient biopsy and PDX samples demonstrated a high degree of similarity, and comparison of successive PDX passages showed few changes in copy number (Figure 3B). Consistent with previous observations [24], the copy number profile of most SCLC tumor models pointed to LOH or copy-neutral LOH affecting 3p, 13q (harboring \textit{RB1}) and 17p (harboring \textit{TP53}) as well as frequent broader chromosomal gains on 3q and 5p (Figure 3B). Notably, MGH1514-1 did not share these canonical genomic characteristics of SCLC tumors, despite confirmed inactivation alterations of \textit{TP53} and \textit{RB1} (Figure 2).

There was an approximate 10-fold range in mutational burdens across the subset of sequenced PDX models, from 27 mutations in MGH1514-1 (<1 mut/Mb) to over 500 mutations in MGH1528-1 (>10 mut/Mb) (Figure 3C). Despite this wide range, we found minimal variation in mutational burden or the distribution of mutation types between patient biopsy and PDX. There was also no significant accumulation of mutations across two PDX passages (<1%). We further analyzed the retention of individual mutations between patient biopsy and PDX models at separate passages. In 5 of the 6 cases (with the exception of MGH1514) at least 95% of all somatic alterations were shared between the tissue biopsy and PDX models (Figure 3D and Supplementary Figure 4). The rare differences between tissue biopsy and PDX samples included an in-frame deletion in \textit{MTOR}, present in the MGH1504 patient tumor biopsy, but not in the CTC-derived PDX; a heterozygous \textit{PIK3CA} splice-site mutation acquired in the MGH1514-1 PDX model; and single-copy gain of the \textit{MYCL1} locus in the MGH1515-1 PDX that may be reflected in the increased transcript levels (Figure 2D, Supplementary Table S5). The fraction of shared mutations between PDX P0 and the patient biopsy
was not significantly different from the fraction shared between P0 and subsequent passages. This held true for both biopsy- and CTC-derived models, indicating that the CTCs collected at the time of the biopsy share the same genomic features as the sampled solid tumor. This result is consistent with the low degree of clonal heterogeneity in SCLC that has been previously reported [22]. In summary, SCLC PDX models retain a stable genome and maintain their somatic alterations between initial model generation (P0) and serial passages (P1/P2) (>95%), thus faithfully recapitulating SCLC patient tumors at the time of model generation.

**Functional fidelity of PDX models to patient response to chemotherapy**

To further assess the capacity of the PDXs to accurately model characteristics of the patient tumor, we sought to quantify their responses to EP. Acquired resistance to EP is commonly observed in the clinic. However, there was no correlation between patient treatment histories and sensitivity to chemotherapeutics *in vitro* across a panel of 63 human SCLC cell lines [42], suggesting inadequacy of the cell lines for modeling clinical behavior. PDXs may better recapitulate patient treatment histories.

To assess EP response in our models, we first optimized an EP regimen to distinguish between serial PDX models derived from the same patient (MGH1518) prior to first line chemotherapy and after subsequent lines of therapy (Figure 4A,B). This regimen consists of cisplatin 7 mg/kg intraperitoneal (IP) day (d)1 and d8 plus etoposide 10 mg/kg IP d1, d2, d3, and d8, d9, d10. Significant tumor shrinkage was induced in the EP-naive model, but not the model derived after the patient had received prior EP, recapitulating the evolving resistance of the patient’s tumor (Figure 4B).
We then applied this EP dosing strategy to 30 PDX models, including 12 treatment-naïve models and 18 post-relapse models (Figure 4C and Supplementary Figure 5). Using large numbers of mice for each model would limit the feasibility of a population-based approach, and recent studies of hematopoietic and solid-tumor PDX model populations have shown that small numbers of animals are sufficient to accurately compare tumor responses [43, 44]. Models were therefore treated in biological duplicate or triplicate, and consistent with these studies, our results show highly concordant tumor volume curves between different mice carrying the same PDX (Figure 4B and Supplementary Figure 5). Responses were quantified by measuring the maximum depth of tumor response (minimum percent initial tumor volume in the days 14-28 window of the treatment), as well as the time to progression (TTP; days to 2x initial tumor volume) (Figure 4A). Response and TTP were strongly correlated across the model panel (Figure 4D), and these metrics were applied to assess the fidelity of the models to patient responses.

To determine whether prior patient exposure to chemotherapy correlated with EP sensitivity in the models, the metrics of PDX response were compared between models derived from EP-naïve versus EP-treated patients (Figure 4E). Maximum tumor response was significantly different, with nearly uniform sensitivity of models from EP-naïve patients, and a range of responses in the models from previously treated patients (Figure 4F). Clinically, first-line chemotherapy is administered for a finite number of cycles, as opposed to ongoing administration until resistance emerges. Therefore, this range of responses in post-relapse models is expected. When we examined TTP as a metric of model sensitivity to EP, we observed a trend toward prolonged TTP in the
models from treatment-naïve patients compared to models from previously-treated patients (Supplementary Figure 6). Notably, assessment of xenograft TTP can be complicated by differences in intrinsic xenograft doubling times (Supplementary Figures 7). To correct for this, the doubling times of each model ($T_{\text{dbl}}$) were calculated for each model in untreated xenografts. Doubling times were uncorrelated with patient treatment history (Figure 4G and Supplementary Figure 8). The corrected TTP (calculated as the ratio of TTP (doubling time in the presence of EP) to intrinsic doubling time; $TTP/T_{\text{dbl}}$ ratio) was significantly different between models derived from EP-naïve vs. EP-treated patients (Figure 4H and Supplementary Figure 8).

We next sought to assess whether the relative TTP of each PDX model was consistent with the TTP of its donor patient. Patient TTP was measured as the number of days from the last dose of EP to the date of first radiographic progression of disease. Although many variables can affect TTP in the clinic, we found that the model EP response was moderately correlated with patient TTP among PDXs derived from previously-treated patients (Figure 4I). This is consistent with the clinical observation that TTP following EP correlates with likelihood of response to next-line DNA-damaging therapy [45]. Clinical TTP data for models from EP-naïve patients was limited to a smaller number of cases, and as a result a thorough comparison to model TTP could not be performed.

Collectively, these results show that PDX responses and TTPs correlate with the patient treatment histories. These results stand in contrast to result from established cell lines, where chemotherapy sensitivity is uncorrelated with patient treatment history [42], and suggest that PDXs more accurately model the clinical behavior of these cancers.
Importantly, they also support the application of PDXs for studying clinically relevant EP-resistance, acquired in patients rather than in an experimental laboratory system.

We therefore next sought to identify potential molecular features that correlate with chemoresistance. Transcriptome sequencing was performed on a subset of 19 models treated with EP, and analyzed for signatures that correlated with treatment sensitivity vs. resistance (Supplementary Table S7). In parallel, quantitative western blots for selected proteins with potential impact on chemoresistance were performed across the 30-model EP cohort. Transcript and protein levels were closely matched for most models in which both could be compared (Figure 4J and Supplementary Figure 9).

Recently, the putative RNA-DNA helicase *SLFN11* has been associated with sensitivity to a number of DNA damaging therapies, including EP [42, 46]. In our PDX cohort, neither *SLFN11* transcript levels (19 models) nor protein levels (30 models) correlated with EP response or TTP (Figure 4K and Supplementary Figure 9). Furthermore, *SLFN11* levels were approximately equivalent in models derived from treatment-naïve and previously-treated patients (Figure 4L and Supplementary Figure 9). A similar lack of correlation with EP response was observed for *ASCL1* and *NEUROD1* (Supplementary Figure 9).

A systematic analysis of the transcriptome sequencing dataset was performed to identify features and pathways that best correlated with EP response (Supplementary Figure 10). The best-correlated transcripts (absolute Spearman coefficient >0.6, 359 genes) were analyzed by gene set enrichment analysis (GSEA, MSigDB v6.0) using only the Hallmark gene sets, a curated collection with small numbers of elements in each set, compiled from multiple independent databases [47, 48]. No gene sets were
positively correlated with sensitivity to EP (FDR q-value cutoff <0.01), reflecting the high stringency of the analysis. However, 10 gene sets scored for EP resistance, including genes upregulated in response to ultraviolet (UV) and reactive oxygen species (ROS) exposure, and genes involved in drug detoxification (“xenobiotic metabolism”) (Figure 4M and Supplementary Table S8).

Also among these were two distinct MYC target lists containing 14 genes in total, which we term the “small MYC” regulon. A MYC target signature was of particular interest given recent data that over-expression of Myc in a SCLC GEMM model promotes tumor growth and may confer relative chemotherapy resistance [49]. Given the number of potential MYC targets that contain a canonical E-box, we performed a secondary assessment of whether these 14 genes represented a MYC transcription signature. We identified the 200 transcripts that most closely mirrored the expression pattern of the small MYC regulon. These transcripts were compared by enrichment analysis (Enrichr) with the ENCODE collection of transcription factor ChIP-seq datasets [50, 51]. 807 ChIP-seq datasets from 181 transcription factors contained at least one overlapping gene with the query set (Figure 4N and Supplementary Table S8). MYC and MAX ChIP-seq datasets were among the most enriched for the 200-gene query set, as well as the MYC family transcription factor USF1, supporting the conclusion that the 14-gene signal initially detected by GSEA does represent a MYC target signature (Figure 4M). Indeed, 155/200 genes in the large MYC set were contained within the top 8 ChIP-seq datasets (Supplementary Table S8). This “large MYC” regulon, composed of E-box containing genes that are directly bound by MYC, was strongly anti-correlated with EP sensitivity, measured both by response and TTP (Figure 4P and Supplementary...
Figure 11). These results support the conclusion that up-regulation of a MYC signature may be a biomarker of EP resistance. Furthermore, this analysis demonstrates that this large panel of PDX models and their quantified EP sensitivities can be applied for novel discovery of transcriptional profiles correlated to chemotherapy sensitivity or resistance.

Correlation between patient and models responses to an experimental therapy

To address the capacity of serial PDXs to model tumor evolution from a single patient over multiple time points, we focused on models derived from MGH1528. The patient was a 58-year-old male who had received several prior lines of therapy (MGH1528, Table 1). He was enrolled onto an ongoing phase 1/2 clinical trial of combination olaparib and temozolomide (OT) in patients with SCLC that has progressed following at least one prior line of chemotherapy (NCT02446704). At the time of enrollment on the clinical trial, the patient had widely metastatic disease including a large left axillary mass (Figure 5A). He was treated with OT and had a partial response by RECIST 1.1 criteria, with a nadir at day 89. He remained on study 6.5 months, but ultimately developed progressive disease.

Serial PDX models were generated from CTCs immediately prior to enrollment onto the OT trial (MGH1528-1) and at the time of relapse (MGH1528-2), which allowed for pharmacological interrogation in vivo with the mouse model and in vitro with PDX derived short-term cell cultures (STCs) (Figure 5B, C). We first assessed the in vivo response of the tumors to the combination treatment with OT. Mice bearing tumors from MGH1528-1 were treated with one cycle of OT, which resulted in dramatic tumor regressions, whereas tumors in vehicle-treated mice progressed rapidly (Figure 5B).
However, the PDX tumors derived after the patient’s progression on OT (MGH1528-2) did not respond to this treatment, and in fact demonstrated similar growth kinetics to the vehicle-treated animals (Figure 5B). These serial CTC-derived PDX models therefore reflected the evolving treatment sensitivities of the patient tumor at the time of CTC-collection.

Although PDX models permit functional analysis of patient drug responses, the requirement for in vivo experiments may limit both throughput and assay variety. Short-term cultures (STCs) could greatly expand the range of analyses of PDX models while generating fewer in vitro artifacts than long-term cell line establishment. STCs were initiated from the MGH1528 serial models and treated with 2-dimensionally titrated combinations of olaparib and temozolomide (Figure 5C). Cultures were initiated on the day of tumor resection, and treated within 24 hours of cell seeding. The MGH1528-1 culture demonstrated high sensitivity to both olaparib and temozolomide, as well as the combination. By contrast, MGH1528-2 was significantly less sensitive to the OT combination (Figure 5C). Thus, OT responses in STCs derived from serial PDX models show concordance with both in vivo responses and the patient clinical course. We anticipate that these types of serial models will enable detailed mechanistic studies of how resistance to therapy evolves in patients.

**DISCUSSION**

Numerous model systems for studying SCLC exist, including cell lines, GEMMs, and PDXs. While each has its relative merits and limitations, the purpose of any model system is to enable clinically relevant and impactful discoveries. Here we report the
efficient production of a large panel of PDX models, and demonstrate the high genomic and functional fidelity of these models when compared with the patient tumors from which they were derived. These findings support a prominent role for PDX models in SCLC translational science. The high efficiency of our PDX development platform (38% for CTCs and 89% for biopsies) suggests that the generation of large model populations, as well as serial models from the same patient, may ultimately become routine, particularly in the context of clinical trials.

Importantly, our experience generating SCLC PDXs from biopsies and effusions demonstrates that model development is highly efficient from a wide variety of metastatic sites (including lymph nodes, subcutaneous nodules, brain metastases, adrenal metastases, and pleural fluid), and using a variety of modalities (including CT guided biopsy, ultrasound guided biopsy, EBUS, surgical resection, thoracentesis, and pericardiocentesis) (Supplementary Table S2). PDX development was more efficient from biopsies than from CTCs, likely due to larger numbers of starting tumor cells and preservation of tumor microenvironments. At MGH, core biopsies at the time of diagnosis are standard, even for suspected SCLC. This provides an opportunity to consent patients to a research protocol prior to their diagnostic biopsy and to collaborate with interventional colleagues to collect tissue for PDX development. At the time of progression after a prior therapy, repeat biopsies are not the standard of care, and thus tissue collection is restricted to those patients undergoing a biopsy as a requirement for a clinical trial or for unusual clinical circumstances. We therefore encourage the incorporation of pre- and post-treatment biopsies into SCLC clinical trials,
as these tissues can be used not only for direct assays, but also for efficient generation of PDX models representative of the disease at the matched time point.

The CTC-iChip or similar automated technologies may further help to standardize the generation of SCLC PDX models, particularly in situations when a biopsy is not otherwise clinically indicated. In addition to our own technology, there are multiple similar technologies currently available including Clearbridge, Apocell, and CytoScale (reviewed in [21]). The CTC-iChip technology is also currently being commercially developed. We anticipate the high efficiency of PDX generation from CTCs will be reproducible with other microfluidic CTC isolation technologies, though this remains to be directly tested.

SCLC tumors have extremely complex genomes with extensive copy number alterations and a high mutational burden. Furthermore, these tumors harbor recurrent alterations that can promote genome instability, most notably inactivation of TP53 and RB1. Initial studies on PDX models of SCLC have confirmed that they share the genomic and molecular hallmarks of the human disease [5, 10, 15]. However, the extent to which an individual model faithfully recapitulates the specific molecular and functional characteristics of the donor patient tumor from has been uncertain.

Here we performed a comprehensive genomic study of SCLC PDX models, which for the first time demonstrates that the somatic mutational landscapes of the models closely match synchronous tumor biopsies, and remain stable over early passages in mice. These features are true of both biopsy- and CTC-derived PDX models, and stand in contrast to other solid tumor types. For malignancies such as lung adenocarcinoma [52], colorectal cancer and melanoma [53, 54], marked genomic
heterogeneity has been observed between anatomically distinct metastases, especially following the emergence of resistance to therapy. For PDX models of breast cancer, retention of intra-tumor heterogeneity has been demonstrated, as well as the evolution of new sub-clones over serial passages [55]. Furthermore, a recent comprehensive study of PDX models derived from diverse tumor types, but excluding SCLC, demonstrated marked genomic evolution over early passages [25]. Therefore the fidelity of the CTC-derived SCLC models was particularly surprising: a small number of tumor cells with high mutational burdens, shed into circulation, collected, grown into xenografts and repeatedly passaged, were found to have nearly superimposable genomes with patient tumor biopsies taken from anatomically distinct locations (Figure 3D). This supports the idea that despite a high mutational burden (>8 mutations per Mb), clonal homogeneity is a distinguishing feature of SCLC [22], and in addition suggests relative genomic stability. The genomic fidelity of CTC-derived models has important implications for the utility of SCLC CTCs in translational research, and for the validity of molecular diagnostics that use live cells and cell-free DNA. We do note that there are handful of somatic alterations that differ between the PDX models and the biopsies (<5%). It remains to be understood how these subtle differences impact on the functional fidelity of the PDX to the patient’s tumor, and further assessments of SCLC PDX models are undoubtedly warranted.

A large panel of PDX models, derived from both treatment-naïve and previously treated patients, enables well-powered functional in vivo studies. Here, among 30 PDX models, we observe a range of sensitivities to standard of care chemotherapy, EP. Unlike in SCLC cell lines [42], EP sensitivity in our PDX models correlates with patient
treatment history, supporting the conclusion that these models more accurately recapitulate the behavior of patients’ tumors. The breadth of this collection of PDXs also enables analysis of correlative transcription signatures. Across 19 models, we identify several expression signatures that correlated with relative EP resistance, many of which warrant further study. Notably among this list, we observe that expression of a subset of MYC targets emerges as a marker of EP resistance. This observation is consistent with recent work in GEMMs, where over-expression of Myc promotes growth of tumors that rapidly relapse after EP treatment in vivo [49]. In GEMMs Myc over-expression drives a Neurod1 high, Ascl1 low (so-called “neuroendocrine-low”) profile, though this dichotomy is less prominent across a panel of human cell lines and tumors. Similarly, we find that some tumors exhibit distinct expression of these two transcription factors (Supplemental Figure 9A), though others do not fit this pattern of mutually exclusive expression. Thus, while the interplay between expression of MYC, NEUROD1 and ASCL1 may be more complex in human tumors than in GEMMs, our findings support the conclusion that a MYC expression signature is a marker of greater chemotherapy resistance in SCLC, and we provide the first demonstration of this observation in unperturbed samples from patients.

SLFN11 has also been described as a potential biomarker of sensitivity to DNA damaging agents [5, 10, 46, 56]. In a study by Gardner et al., acquired EP resistance in PDX models derived from chemotherapy-naive patients led to down-regulation of SLFN11. By contrast, we found SLFN11 expression levels (mRNA or protein) did not correlate with either PDX EP response or history of prior chemotherapy exposure for the donor patients. These differences likely arise from the fact that these are orthogonal
experiments; one testing the effect of induced high-level EP resistance within the same model, and the other comparing expression with intrinsic chemotherapy response and clinical history. Additional work is warranted to elucidate the role of *SLFN11* and its potential applications, but in our dataset it was not a biomarker of EP sensitivity.

In summary, we introduce here a new strategy for efficient development of a panel of SCLC PDX models, validate the genomic and functional fidelity of these models, and use the panel to assess markers of chemotherapy response. We believe that these advances lay the foundation for further functional analyses across large panels of SCLC PDX models in which experimental results can be directly compared to patient clinical outcomes. Additionally, reliably efficient generation of PDX models enables generation of isogenic models from SCLC patients at multiple times in their treatment course. We anticipate that these types of models will be a powerful resource in the context of SCLC drug development, facilitating the identification of biomarkers and mediators of sensitivity and acquired resistance to therapy.

**MATERIALS AND METHODS**

Extended methods are available in the supplementary material.

*PDX model generation.* All tissue and blood samples from patients were collected per Institutional Review Board (IRB) approved protocols with written informed consent from the patients and in accordance with the Declaration of Helsinki. All mouse studies were conducted through IACUC approved animal protocols in accordance with Massachusetts General Hospital institutional guidelines. To initiate a PDX model (P0), SCLC tumor material (CTCs, leukocyte/RBC-depleted effusion, or tumor core needle
biopsy or surgical sample) was resuspended in 1:1 ice-cold HITes media and Matrigel (Corning), and injected subcutaneously via large bore (18G) needle into the right flank of an NSG mouse (NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ, Jackson Laboratories). Whole blood and pleural or pericardial effusions were collected and transported at room temperature, and core biopsy samples on ice. Biopsy samples were diced by scalpel prior to resuspension and injection. Effusion samples were RBC-depleted (BioLegend RBC lysis buffer) and leukocyte depleted (Miltenyi Biotec anti-CD45 IgG microbeads) per standard protocols. SCLC CTCs were enriched from fresh peripheral blood samples using either the CTC-iChip microfluidic device, as described previously for negative depletion of leukocytes and enrichment of untagged CTCs [27, 28], or the manual Ficoll gradient method previously described [20]. After tumor emergence, palpable tumors were measured with electronic calipers weekly until tumors exceeded 1500 mm<sup>3</sup>, at which point animals were euthanized and tumors were resected. Scalpel-dissected xenograft fragments were either immediately implanted into NSG mice for passaging, cryopresereved for later passaging, fixed in 10% neutral buffered formalin (Sigma) for pathologic analysis, or fresh-frozen in liquid nitrogen for molecular analysis. For pathologic review, 5 µm sections of FFPE tissue were stained with hematoxylin and eosin as well as antibodies against chromogranin, synaptophysin, CD56, CD45, and Rb (antibody details in supplementary methods). The histologic diagnosis of small cell carcinoma was rendered in accordance with WHO Classification of the Lung, Pleura, Thymus and Heart, 4<sup>th</sup> edition [57].

**Whole Exome Sequencing (WES) and Transcriptome Sequencing.** Total DNA and RNA were isolated from fresh-frozen or FFPE tumor tissue, and germline DNA was
obtained from matched normal donor blood or FFPE tissue histologically confirmed to be free of tumor cells. DNA from FFPE tissue was extracted with the Maxwell FFPE DNA purification kit on a Maxwell 16 MDx instrument (Promega). Nucleic acids were extracted from fresh-frozen tissue and patient-derived blood by standard protocols (supplementary methods). For WES, DNA was fragmented by sonication, end-repaired and adaptor ligated with incorporation of index barcodes, size-selected and enriched with Sure select XT (Agilent), and sequenced with a paired end 2x75 bp protocol for an average coverage of 100-120x (Supplementary Table S3). For paired-end RNA-seq, cDNA libraries were prepared with the Illumina TruSeq kit and sequenced with a paired-end 2x75 bp protocol on an Illumina HiSeq instrument. For single-end RNA-seq, cDNA libraries were prepared with the Kapa Stranded RNA-seq kit with RiboErase HMR method and sequenced with a single-end 75 bp protocol on a NextSeq 500 instrument. Sequence alignments were performed against both human and mouse reference genomes to filter mouse-specific reads. Somatic mutations and copy number alterations were determined as previously described [22, 24]. Transcript expression levels were determined using Cufflinks and expressed as reads per kilobase million (RPKM) (paired-end RNA-seq) or using Kallisto and expressed as transcripts per million (TPM) (single-end RNA-seq). Downstream bioinformatic analyses are described in the supplementary information. Paired-end whole exome and transcriptome sequencing data is deposited at the European Genome-phenome Archive, which is hosted by the EBI (EGA, http://www.ebi.ac.uk/ega/), under accession number EGAS00001002853. Single-end transcriptome sequencing data is deposited in the NCBI’s Gene Expression

**Immunoblotting.** Fresh-frozen xenograft samples were lysed in RIPA buffer using a TissueLyzer II (Qiagen) homogenizer. SDS-PAGE was performed by standard methods, and PVDF membranes were probed with the following antibodies: from Cell Signaling Technology: alpha-tubulin, beta-actin, NeuroD1, total EGFR, p-AKT (S473), p-ERK1/2 (T202/Y204); from Abcam: Ascl1 (MASH1); from Santa Cruz Biotechnology: Sfln11. Membranes were imaged with a Syngene G:BOX and band densitometry was performed using Syngene GeneSys software. Ratio to loading control (alpha-tubulin) was calculated, and lysates from established SCLC cell lines (CORL88, CORL279, NCIH82, NCIH1048, DMS273) were used as inter-blot standards. Cell lines were obtained between 2015-2017 from the Massachusetts General Hospital Center for Molecular Therapeutics, which performs routine authentication by SNP and STR analyses, and were passaged in HITES media + 2% FBS for less than 3 months prior to lysate preparation.

**Mouse treatment studies.** Trials were initiated at xenograft volumes = 400-600 mm$^3$ for 3-5 mice per model per treatment arm, and tumors were measured 2-3x weekly. EP: cisplatin 7 mg/kg intraperitoneal (IP) d1,8 + etoposide 10 mg/kg IP d1-3,8-10. OT: olaparib 50 mg/kg oral gavage (OG) d1-5 + temozolomide 25 mg/kg OG d1-5. EP trial tumor metrics: TTP = days from start of treatment to 2x initial tumor volume (ITV), response = change in tumor volume between ITV and d14-28 minimum, TTP/T$_{dbl}$ = ratio of TTP to tumor doubling time in untreated mice. Endpoints: tumor volume > 2x ITV or 80 days after start of treatment.
**PDX short-term cultures.** Xenografts were resected, fragmented, and rapidly dissociated with a gentleMACS Octo Dissociator (Miltenyi Biotec). Live cells were enriched by Ficoll gradient, and depleted of murine cells with anti-mouse IgG microbeads (Miltenyi Biotec). PDX culture suspension were seeded in 96-well format in HITES media + 2% FBS + 10 µM ROC kinase inhibitor (Y-27632, Selleckchem). Titration of olaparib and temozolomide was performed with a D300e digital drug dispenser (Tecan Life Sciences). Viability was assessed after 5 days using CellTiter-Glo (Promega).

**ACKNOWLEDGEMENTS**

We are grateful to the patients and families who participated in these research studies. We thank Jeffrey Engelman for his vision and guidance in launching this project. We thank M. Stanzione, I. Sanidas, A. Guarner-Peralta, B. Krishnan, K. Tschop, W. Miles, B. Nicolay, and current and former members of the Dyson and Farago research groups; L. Zou, R. Corcoran and C. Benes for critical discussions and scientific input; and J. Sullivan for guidance on CTC isolation. We thank the regional computing center of the University of Cologne (RRZK) for providing the CPU time on the DFG-funded supercomputer ‘CHEOPS’ as well as the support. We thank the members of the MGH thoracic oncology group and other MGH Cancer Center staff for assistance with recruitment of patients and collection of samples.
REFERENCES


<table>
<thead>
<tr>
<th>Model</th>
<th>PDX type\textsuperscript{a}</th>
<th>Biopsy to model initiation (days)\textsuperscript{b}</th>
<th>P0 latency (days)\textsuperscript{c}</th>
<th>Patient clinical stage</th>
<th>Patient prior therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH1504-1</td>
<td>CTC</td>
<td>3</td>
<td>160</td>
<td>LS</td>
<td>none</td>
</tr>
<tr>
<td>MGH1512-1</td>
<td>biopsy</td>
<td>0</td>
<td>60</td>
<td>ES</td>
<td>EC, irinotecan</td>
</tr>
<tr>
<td>MGH1514-1</td>
<td>CTC</td>
<td>4</td>
<td>130</td>
<td>ES</td>
<td>none</td>
</tr>
<tr>
<td>MGH1515-1</td>
<td>CTC</td>
<td>8</td>
<td>138</td>
<td>ES</td>
<td>none</td>
</tr>
<tr>
<td>MGH1518-1</td>
<td>biopsy</td>
<td>0</td>
<td>81</td>
<td>ES</td>
<td>none</td>
</tr>
<tr>
<td>MGH1525-1</td>
<td>CTC</td>
<td>1</td>
<td>45</td>
<td>ES</td>
<td>none</td>
</tr>
<tr>
<td>MGH1528-1</td>
<td>CTC</td>
<td>--</td>
<td>107</td>
<td>ES</td>
<td>EC, topotecan, EC, paclitaxel, exp1, exp2, navelbine</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of PDX models selected for whole exome sequencing.  
\textsuperscript{a}PDX type indicates if the model is CTC-derived or biopsy-derived.  \textsuperscript{b}For all CTC-derived models except MGH1528-1, a biopsy was collected near the time of CTC collection, without any intervening therapy, and these were the patient tumor samples used for WES. Time from biopsy to model initiation is shown. \textsuperscript{c}P0 latency indicates time from tissue implantation to when a tumor was first palpated on the flank of the recipient mouse. Abbreviations: LS, limited stage; ES, extensive stage, EC, carboplatin and etoposide; exp, experimental therapy on clinical trial.
FIGURE LEGENDS

Figure 1. Generation of a population of SCLC PDX models. (A) Strategy for SCLC PDX development. PDX models were initiated from whole blood via CTC isolation (red, top), core tumor biopsies (blue, bottom) or effusion specimens. Whole blood samples were processed via the CTC-iChip<sup>neg</sup> device, which enriches CTCs in a three-step process: (1) separation of non-nucleated cells and plasma by size hydrodynamic diameter using a microarray of posts, (2) inertial focusing through an asymmetric serpentine channel to position cells in a single line, and (3) negative selection of leukocytes decorated with anti-CD45/CD66b magnetic beads by magnetic separation (yellow arrow = magnetic deflection). Biopsy, effusion and CTC samples were injected SC into the flanks of NSG mice, monitored for tumor emergence (P0 latency), and then serially passaged (P1, P2). Tumor samples were obtained for molecular and pathologic analysis and for cryopreservation of the model. (B) Panel of SCLC PDX models with abstracted patient clinical courses. Models derived from either CTCs (red circles) or biopsies/effusions (blue circles) were generated at various time points throughout the treatment of the patient (arrows). Arrows are not drawn to scale with respect to time on treatments. (C) Latency to (P0) tumor emergence for models initiated June 2014 – June 2016. (D) Efficiency of PDX generation from CTCs and biopsies/effusions. Total attempts in gray, successful in color. (E) Pathologic confirmation of SCLC. Shown are SCLC histology (H&E staining) comparison between biopsy and PDX derived from either CTCs (MGH1504-1) or biopsy (MGH1512-1), as well as immunohistochemical (IHC) stains for neuroendocrine markers and of nuclear Rb1. Direct comparison of histology and IHC stains in a patient sample and corresponding CTC-derived PDX.
model (MGH1515-1) are also shown. Additional examples are shown in Supplementary Figure 1. H&E, hematoxalin and eosin; Chrg., chromogranin; Syp., synaptophysin.

**Figure 2. Genomic alterations and expression profiles in SCLC PDX models.** (A) Spectrum of genomic alterations in the panel of 7 PDX models. Top panel: Bi-allelic genomic inactivation of *TP53* and *RB1*. Lower panel: Notable alterations in PDX models beyond *TP53* and RB1 referring to previously identified significantly mutated genes in SCLC (*) [22] and to mutated cancer census genes of therapeutic relevance (#). The bottom panel displays the type of base-pair substitution referring to the representative data of PDX P0 (Supplementary Table S2). (B) Detection of the out-of-frame fusion transcript *TP53-ITNL2* in MGH1514-1 by paired-end RNA-seq. (C) Pearson correlation matrix for passage 0 and passage 1-2 tumors from each model, using genes with highly variable transcription levels across all samples (max RPKM >3, coefficient of variation >1, 1568 transcripts). Source of each PDX model (CTCs, C; biopsy, B) is indicated in parentheses next to the model number. (D) Clustering analysis on transcriptome sequencing data of PDX models (n=13 from 7 patients) and human SCLC tumors (n=20, from George *et al.*) selected to represent the neuroendocrine-high and low groups as previously described. Clustering performed on genes that distinguish human primary tumors to avoid signatures associated with human immune and stromal infiltrates. All data processed with RNA-seq pipeline for human+mouse reads.

**Figure 3. High genomic fidelity of SCLC PDX models derived from both CTCs and biopsies.** (A) Comparative genomic analysis on patient biopsy vs. PDX P0 and
subsequently passaged PDX tumors (P1 for MGH1514-1 or P2 for all other models). (B-C) Analysis of the copy number alteration status (B), and of the number and type of somatic mutations (C) is displayed for 6 models. Initial tumor biopsy and derivative PDX models are described according to the color panel provided in (A). (D) Venn diagrams show overlap of mutations between patient biopsy, PDX P0 and PDX P1/2 exomes. Diagrams are colored according to the annotation in (A) and are scaled to total number of mutations. Number of private mutations not shared by all 3 samples is shown to side of the diagrams, with color bar below indicating the sample(s). Source of each PDX model (CTCs, C; biopsy, B) is indicated in parentheses next to the model number.

**Figure 4. SCLC PDX model responses to first-line chemotherapy reflect patient treatment histories.** (A) % Initial tumor volume (ITV) vs. days after EP start for a single xenograft treated with two one-week cycles of cisplatin 7 mpk IP d1 + etoposide 10 mpk IP d1-3 (tan bars). Response = minimum %ITV between d14-28. Time to progression (TTP) = time to 2x ITV. (B) Differential EP response of serial models from patient MGH1518 derived before 1st line chemotherapy and after 2nd line therapy. (C) Trial of EP across a population of 30 PDX models: 12 from treatment-naïve patients (green) and 18 from previously treated patients (purple). Results presented in D-O, with same green/purple color code in E-I and L. (D) Correlation of PDX EP response and TTP (E) Waterfall plot of PDX best response. (F-H) Comparison of tumor metrics following EP treatment in PDX models from treatment-naïve vs. post-relapse patients, with unpaired T-test p-values: best response (F), doubling time (G), and ratio of TTP to doubling time (H). (I) TTP in post-relapse PDX models vs. EP TTP in the donor patients. (J)
Correlation of SLFN transcript abundance in transcriptome sequencing (transcripts per million, TPM), and protein levels measured by quantitative western blot (arbitrary units) across 19 models, with logarithmic trend line. **(K)** Lack of correlation between EP response (rank 1 = deepest response) and *SLFN11* expression (rank 1 = highest level): protein on left (30 models), transcript on right (19 models). **(L)** No difference in *SLFN11* protein levels between PDX models from treatment-naïve vs. post-relapse patients. **(M)** Gene set enrichment analysis (GSEA) of transcripts that correlate with PDX EP resistance (Spearman ρ > 0.6) using “Hallmark” gene sets (MSigDB v6.0). Gene sets with false discovery rate (FDR) of less than 1% are shown. **(N)** 200 putative MYC targets that correlate with GSEA MYC signature were compared with inventory of ChIP-seq datasets. 807 datasets from ENCODE, covering 181 transcription factors (TFs), had >1 intersecting gene. Inset: top enriched TFs for these genes, with a Kolmogorov–Smirnov (KS) statistic p-value < 0.01. **(O)** MYC regulon correlates with EP resistance. MYC regulon = 155/200 putative MYC targets that were present in top 7 MYC/MAX ChIP-seq dataset. Regulon expression rank vs. EP response rank for 19 PDX models.

**Figure 5. SCLC PDX models recapitulate patient responses to an experimental therapy.** **(A)** Axial computed tomography (CT) scan images from patient MGH1528 at multiple time points: immediately before starting treatment on olaparib + temozolomide (OT, left), during treatment at nadir of response (middle), and at the time of progression (right). The schematic above indicates prior lines of therapy, with carboplatin + etoposide (EC) shown in black arrows, other therapies shown in gray arrows, and OT shown as an orange arrow. Arrows are not drawn to scale with respect to time on
treatments. (B) PDX models generated from patient MGH1528 prior to OT (MGH1528-1) and at the time of progression (MGH1528-2) were treated with OT (blue) or vehicle (gray) for one cycle (5 days, blue shading). Tumor dimensions were measured three times per week and plotted as percent initial tumor volume (ITV) vs time. (C) Short-term cultures (STCs) generated from untreated PDX tumors were treated with OT combinations in vitro. Cultures were seeded on the day of tumor extraction (day 0), treatment was initiated within 24 hours (day 1), and viability was assayed after 5 days of treatment (day 6). Olaparib doses (9) range from 10 nM – 10 μM and temozolomide doses (5) from 1 μM – 300 μM, both on exponential scales.
Figure 1

A

Magnetic Beads (CD45, CD66b)

1. Size Separation

2. Inertial Focusing

3. Magnetic Separation

SCLC CTCs

Core Biopsy
(if available)

Rx

Relapse:
Repeat CTC collection and model generation as above

NSG mouse

SCLC Tumor

Stroma

B

Model # | Patient Course
---|---
MGH1501 | 
MGH1504 | 
MGH1505 | 
MGH1506 | 
MGH1508 | 
MGH1512 | 
MGH1514 | 
MGH1515 | 
MGH1517 | 
MGH1518 | 
MGH1520 | 
MGH1521 | 
MGH1522 | 
MGH1523 | 
MGH1524 | 
MGH1525 | 
MGH1528 | 
MGH1529 | 
MGH1534 | 
MGH1535 | 
MGH1536 | 
MGH1537 | 
MGH1538 | 
MGH1541 | 
MGH1542 | 
MGH1543 | 
MGH1545 | 

C

<table>
<thead>
<tr>
<th>P0 latency (wks)</th>
<th>No. of models</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

D

<table>
<thead>
<tr>
<th>P0 Latency</th>
<th>iChip</th>
<th>Bx/Eff</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0 attempts</td>
<td>38%</td>
<td>31%</td>
</tr>
<tr>
<td>P0 generated</td>
<td>62%</td>
<td>69%</td>
</tr>
</tbody>
</table>

E

Patient | PDX | Chrg. | Syp. | Rb
---|---|---|---|---
MGH1504-1 | 
MGH1512-1 | 

H&E | Chrg. | Syp. | CD56 | CD45 | Rb
---|---|---|---|---|---
Patient | 
PDX | 

Downloaded from cancerdiscovery.aacrjournals.org on February 27, 2018. © 2018 American Association for Cancer Research.
Figure 3

A

Patient biopsy vs. PDX P0, PDX P1, PDX P2

B

1 3 5 7 9 11 13 15 17 19 21

MGH1504-1
MGH1512-1
MGH1514-1
MGH1515-1
MGH1518-1
MGH1525-1
MGH1528-1

Integral Copy Number (iCN)

< 0.3 2.0 >6.0

C

Mutations

MGH1504-1 MGH1512-1 MGH1514-1 MGH1515-1 MGH1518-1 MGH1525-1 MGH1528-1

D

Drapkin et al.

MGH1504-1 (C) 14
10 281

MGH1512-1 (B) 10
1 159

MGH1514-1 (C) 6 11
11 21

MGH1515-1 (C) 6 5
1 110

MGH1518-1 (B) 4
1 308

MGH1525-1 (C) 7 9
7 194

Patient biopsy ∩ PDX P0

PDX P0 ∩ PDX P1

PDX P0 ∩ PDX P2
Figure 5

A. MGH1528 Clinical Course

- EC → OT
- Baseline → Day 89: Nadir → Day 158: Progression

B. MGH1528 CTC-derived models

- MGH1528-1 Pre-OT: OT treatment window → OT-treated tumor volume → vehicle ctrl. tumor volume
- MGH1528-2 Post-OT

C. MGH1528 short term cultures

- MGH1528-1: Olaparib 300 μM
- MGH1528-2: Olaparib 1 μM

Viability (% no drug):
- 100%
- 50%
- 0
Genomic and functional fidelity of small cell lung cancer patient-derived xenografts

Benjamin J. Drapkin, Julie George, Camilla L. Christensen, et al.

Cancer Discov  Published OnlineFirst February 26, 2018.

Updated version
Access the most recent version of this article at:
doi:10.1158/2159-8290.CD-17-0935

Supplementary Material
Access the most recent supplemental material at:
http://cancerdiscovery.aacrjournals.org/content/suppl/2018/02/24/2159-8290.CD-17-0935.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerdiscovery.aacrjournals.org/content/early/2018/02/24/2159-8290.CD-17-0935.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.