Epigenetic Therapeutic Strategies to Target Molecular and Cellular Heterogeneity in Pancreatic Cancer

Lennart Versemann\textsuperscript{a} Elisabeth Hessmann\textsuperscript{a,b} Maria Ulisse\textsuperscript{a,b}

\textsuperscript{a}Department of Gastroenterology, Gastrointestinal Oncology and Endocrinology, University Medical Center Goettingen, Goettingen, Germany; \textsuperscript{b}Clinical Research Unit KFO5002, University Medical Center Goettingen, Goettingen, Germany

Keywords
Epigenetics · Pancreatic cancer · Subtype · Tumor heterogeneity

Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) remains a major challenge in cancer medicine and is characterized by a 5-year survival rate of <10\%. Compelling evidence suggests that the devastating disease outcome of PDAC patients is linked to a high degree of intra- and interindividual tumor heterogeneity, which is predominantly installed at the level of gene transcription. The cellular and molecular complexities of the disease explain the poor efficacy of “one-size-fits-all” therapeutic approaches in PDAC treatment and strongly argue for pursuing tailored therapeutic strategies to tackle PDAC. In a highly dynamic manner, a network of transcription factors and epigenetic regulatory proteins temporally and spatially control the diverse transcriptomic states determining PDAC heterogeneity. Given the reversibility of epigenetic processes, pharmacological intervention with key epigenetic drivers of PDAC heterogeneity appeals as a promising concept to shift the transcriptomic phenotype of PDAC toward a less aggressive and more chemosensible state. Summary: In this review, we discuss the chances and pitfalls of epigenetic treatment strategies in overcoming and shifting molecular and cellular PDAC heterogeneities in order to combat PDAC. To this end, we utilized the keywords “pancreatic cancer,” “heterogeneity,” and “epigenetics” to search for relevant articles on the database PubMed and selected interventional studies enrolling PDAC patients as displayed in clinicaltrails.gov to generate a synopsis of clinical trials involving epigenetic targeting. Key Messages: Targeting epigenetic regulators in PDAC represents a promising concept to reprogram molecular and cellular tumor heterogeneities in the pancreas and hence to modulate the PDAC phenotype in favor of a less aggressive and more therapy susceptible disease course. However, we just start to understand the complex interactions of epigenetic regulators in controlling PDAC plasticity, and a clinical breakthrough utilizing epigenetic targeting in PDAC patients has not been achieved yet. Nevertheless, increasing translational efforts which consider the pleiotropic effects of targeting epigenetic regulation in different cellular compartments of the tumor and that focus on the utility and sequence of combinatory treatment approaches might pave the way toward novel epigenetic treatment strategies in PDAC therapy.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) remains a major challenge in cancer medicine. Indeed, despite remarkable efforts in translational research and drug development, the overall 5-year survival rate of <10\% of patients has remained unchanged for almost 20 years [1]. Major causes for the dismal outcome are the exceptionally aggressive tumor biology with early onset of metastasis and the remarkable resistance to conventional chemotheraphy. Tumor cell progression and therapy evasion
processes are driven by a high degree of cellular and molecular tumor heterogeneity [2]. Increasing evidence suggests that PDAC heterogeneity is determined by transcriptomic phenotypes, which are hierarchically installed and controlled by epigenetic cues [2–8]. Epigenetic regulatory proteins converge on the transcriptional landscape by controlling, for example, chromatin accessibility, DNA methylation, and histone modification, thus fine-tuning the transcriptional output of a given cell in a spatially and temporally restricted manner [9]. The dynamic nature and the reversibility of epigenetic processes characterize epigenetic regulatory proteins as promising targets to shift transcriptional phenotypes of cancer cells toward less aggressive and more therapy susceptible states. In this review, we discuss conceptual and translational efforts exploiting epigenetic targeting for PDAC treatment with a particular focus on the consequences of epigenetic reprogramming on the molecular and cellular heterogeneity of the disease.

**Targeting Epigenetics to Interfere with Molecular PDAC Heterogeneity**

**Molecular PDAC Subtypes**

Given the advances in next-generation sequencing technologies and motivated by the successes of molecular stratification-based treatment approaches in other tumor entities [10–12], the last decade has witnessed a plethora of whole-genome sequencing studies and transcriptional profiling analyses conducted in large cohorts of PDAC tumors aiding at the dissection of the molecular landscape of PDAC [2–8, 13]. These studies did not only reaffirm signature mutations in KRAS, TP53, CDKN2A, and SMAD4 but led to the identification of numerous additionally mutated or transcriptionally altered genes. Importantly, the molecular heterogeneity of PDAC is reflected in the identification of various molecular and phenotypic PDAC subtypes with prognostic and therapy predictive significance [3–5, 14]. Despite discrepancies in the definition of molecular PDAC subtypes, transcriptome and epigenome analyses consistently identified 2 major lineages which separate PDAC into basal-like (also called “squamous” or “quasi-mesenchymal”) and classical (also considered as “progenitor-like”) subtypes [2–8, 13, 14]. While the basal-like subtype is associated with a high tumor grade, strong chemoresistance, and the worst prognosis [3–5, 14], classical subtype tumors are better differentiated, associated with improved responsiveness to chemotherapy and a better prognosis [3, 4, 14]. Molecularly, these subtypes are linked to distinct gene signatures and epigenetic profiles [8, 15]. While the basal-like subtype shows a more mesenchymal expression profile, the classical subtype comprises an epithelial differentiation gene signature [2–8, 13, 14]. Moreover, the 2 subtypes differ in the activity of distinct superenhancers and their upstream regulators [8]. Superenhancers operate as regulatory elements known to have a huge ability to influence target gene expression, to have cell- and state-specific activities, and to be bound by lineage-defining transcription factors [16, 17]. The most prominent transcription factors regulating subtype-specific superenhancers and transcription programs are GATA6, PDX1, and HNFs for the classical, and MET, MYC, and the ΔN isoform of the transcription factor TP63 (ΔNp63), for the basal-like state [8, 15, 18–20]. Importantly, compelling evidence suggests that epigenetic regulators complement and control the activity of these subtype-determining transcription factors, either by influencing their expression or by acting as transcriptional co-regulators [20, 21].

Given the dynamic character of these drivers of PDAC subtype identity, the distinct subtype states are not permanently installed but underlay a high degree of plasticity. Considering the better prognosis and the increased chemosensitivity of classical versus basal-like PDAC subtypes [3–5, 14], the concept of subtype switching seems to be a highly appealing strategy, for example, preceding cytotoxic PDAC therapy. Consistent with this idea, many translational approaches in PDAC aim at deciphering strategies to induce subtype switching. Given the reversibility of epigenetic regulations, pharmacological interference with epigenetic key regulators of PDAC subtype identity has moved into the focus of approaches aiding at molecular PDAC heterogeneity.

**Epigenetic Targeting Strategies to Induce Subtype Switching in PDAC**

Above others, the endodermal lineage transcription factor GATA6 has been characterized as a hierarchical regulator of classical PDAC subtype identity [4, 8, 22] and as a robust surrogate biomarker for differentiating classical (GATA6<sup>high</sup>) and basal-like (GATA6<sup>low</sup>) PDAC subtypes [14]. Consistently, depletion of GATA6 is necessary to induce the basal subtype-specific transcription factor ΔNp63 and to enforce a basal-like PDAC subtype state [22]. Hence, pharmacological approaches inducing or stabilizing GATA6 expression qualify as promising strategies to push PDAC cells toward the classical PDAC subtype. The potential of GATA6 induction for enforcing classical PDAC subtype identity was introduced by a study of Lomberk et al. [8]. Herein, the authors demonstrate that inhibition of the basal-like subtype-specific superenhancer regulator MET induces a basal-like-to-classical subtype switch via transcriptional activation of GATA6 and subsequent induction of GATA6-dependent gene regulation [8]. Along the same line of evidence, loss of the basal-like subtype-specific transcription factor GLI2, which is involved in Hedgehog signaling [23], re-
Epigenetic Treatment in Pancreatic Cancer

Epigenetic Treatment in Pancreatic Cancer

Visc Med 2022;38:11–18
DOI: 10.1159/000519859

sults in increased GATA6 expression and acquisition of classical gene signatures in PDAC. While these reports emphasize the role of subtype-specific transcription factors in regulating the expression of their classical counterpart GATA6, Patil et al. [21] recently identified the histone methyltransferase enhancer of zeste homolog 2 (EZH2) as a direct transcriptional regulator of GATA6 in PDAC. In accordance with its activity as a transcriptional repressor, EZH2 binding to the GATA6 TSS region silenced GATA6 transcription, thus promoting PDAC invasion and metastasis. Interestingly, blockade of EZH2 activity was sufficient to reinstall GATA6 expression and to induce gene signatures associated with the classical PDAC subtype state. Hence, these data reveal pharmacological interference with EZH2-dependent GATA6 repression as a promising strategy to induce subtype switching, restrain tumor progression, and increase chemosensitivity in PDAC (Fig. 1) [21]. However, given the existence of PDAC tumors with EZH2-independent GATA6 regulation [1], EZH2 targeting might only be beneficial in a subgroup of GATA6-low PDAC subtypes, thus arguing for molecular stratification of GATA6 expression prior to application of EZH2 inhibitors. Nevertheless, the recent FDA approval of the EZH2 inhibitor tazemetostat for the treatment of advanced epithelioid sarcoma and a clinical trial exploring the drug in solid tumor entities including PDAC (NCT04705818, Table 1) suggest a potential clinical relevance of the identified EZH2-GATA6 axis in PDAC (Fig. 1).

In addition to a simultaneous loss of GATA6, epigenetic silencing of HNF4A and HNF1A, has been lately reported as a prerequisite for basal-like subtype identity

Fig. 1. Epigenetic targeting strategies to tackle PDAC subtype identity. PDAC can be classified into the classical and basal-like subtypes differing in chemosensitivity and prognosis. However, there is a dynamic plasticity between these 2 PDAC subtypes. Therefore, forcing a subtype switch from the aggressive basal-like subtype into the less aggressive classical subtype might be a compelling therapeutic option. (1) Derepression of GATA6 expression upon EZH2 inhibition (e.g., using tazemetostat) [21]; (2) BET inhibition (e.g., using JQ1) in KDM6A-deficient PDAC [20]; and (3) GSK3β inhibition (e.g., using TDZD-8) PDAC subtypes characterized by low expression of GATA6/HNF4A [26]. (4) Prospectively, it can be hypothesized that targeting superenhancer activity or chromatin accessibility might be an appealing approach to target PDAC subtype identity upon molecular stratification of the tumor [8, 26, 57]. Designed with https://smart.servier.com/. PDAC, pancreatic ductal adenocarcinoma; BET, bromodomain and extra-terminal motif; DNMT, DNA methyltransferase.
[22]. Loss of HNF4A favors upregulation of GSK3β, which promotes metabolic programs in accordance with the basal-like PDAC subtype [25]. Interestingly, basal-like PDAC subtypes were more sensitive to pharmacological inhibition of GSK3β (Fig. 1) [26]. However, the fact that a subgroup of PDAC developed resistance toward GSK3β inhibition [26] suggests the existence of subgroups within the basal-like PDAC subtype state, whose therapeutic utilities remain to be further explored.

Subtype switching induced by interfering with the epigenetic landscape in PDAC has recently been reported for a subgroup of basal-like PDAC subtypes harboring mutations in the gene encoding for the histone demethylase KDM6A. Mechanistically, loss of KDM6A activity results in aberrant activation of superenhancers that regulate the ΔNp63, RUNX, and MYC oncogenes, thus fostering de-differentiation and metastasis [20]. Importantly, the increased superenhancer activity renders KDM6A-deficient PDAC more susceptible to inhibition of bromodomain and extra-terminal motif (BET) proteins, which reverses basal-like differentiation and restrains PDAC growth both in vitro and in vivo (Fig. 1) [20]. Using preclinical PDAC models, BET-protein inhibition combined with blockade of histone deacetylase (HDAC) activity has been previously introduced as a promising concept in PDAC treatment [27], albeit without considering the PDAC subtype state. Hence, the stratification for KDM6A might even increase the efficacy of BET inhibition for PDAC treatment.

Together, interference with epigenetic regulators to target subtype-specific pathways and expression profiles offers strategies to conquer drug resistance and improve the outcome of PDAC patients. However, the safe and effective application of epigenetic targeting strategies to interfere with PDAC subtype identity requires a more detailed picture reflecting the entire complexity of PDAC molecular heterogeneity. Moreover, regulation of molecular PDAC subtype identity is not restricted to the epithelial tumor cell but is highly influenced by the interaction with other cellular compartments of the tumor.

### Targeting Epigenetics to Interfere with Cellular PDAC Heterogeneity

The cellular heterogeneity of PDAC is based on a pronounced tumor microenvironment (TME) that already forms during pancreatic carcinogenesis and evolves during tumor progression [28]. The PDAC TME, which makes up to 90% of the tumor bulk, comprises cellular (e.g., fibroblasts and immune cells) and acellular components (e.g., collagen and hyaluronic acid), and intensive biochemical interactions exist between these different compartments and the epithelial tumor cells [29, 30].

| NCT number  | Status                        | Drug              | Co-treatment                               | Target                  | Phase | Enrolled tumor entities                        |
|-------------|-------------------------------|-------------------|--------------------------------------------|-------------------------|-------|-----------------------------------------------|
| NCT03264404 | Recruiting                    | Azacitidine       | Pembrolizumab                              | DNMT1                   | 2     | Pancreatic cancer                             |
| NCT01845805 | Active, not recruiting        | Azacitidine       | Abraxane, gemcitabine                      | DNMT1                   | 2     | Pancreatic cancer                             |
| NCT04257448 | Recruiting                    | Azacitidine, romidepsin | Nab-paclitaxel + gemcitabine, durvalumab + lenalidomide capsule | DNMT1, HDAC class I   | 1/2   | Pancreatic cancer                             |
| NCT03250273 | Active, not recruiting        | Entinostat        | Nivolumab                                  | HDAC class I            | 2     | Pancreatic cancer, cholangiocarcinoma         |
| NCT01638533 | Active, not recruiting        | Romidepsin        | None                                       | HDAC class I            | 1     | Pancreatic cancer and other solid tumor entities, hematological malignancies |
| NCT04705818 | Not yet recruiting            | Tazemetostat      | Durvalumab                                  | EZH2                    | 2     | Pancreatic cancer and other solid tumor entities |
| NCT02349867 | Active, not recruiting        | Vorinostat        | Gemcitabine + sorafenib + chemoradiation    | HDAC class I/II         | 1     | Pancreatic cancer                             |
| NCT03878524 | Recruiting                    | Vorinostat        | 52 drugs (chemotherapy, small inhibitors, antibodies) | HDAC class I/II         | 1     | Pancreatic cancer and other solid tumor entities, hematologic malignancies |

PDAC, pancreatic ductal adenocarcinoma; DNMT, DNA methyltransferase; HDAC, histone deacetylase. Selection criteria applied in clinicaltrials.gov: interventional study type, pancreatic cancer, epigenetic treatment involved.
Importantly, the TME does not only promote PDAC progression, but it also significantly impacts on PDAC therapy response. Indeed, acellular and cellular components of the PDAC stroma significantly reduce the exposure of tumor cells to chemotherapeutic agents, for example, by causing a hypovascular microenvironment and by scavenging of active chemotherapeutic metabolites, respectively [29, 31–33]. Moreover, the immune cell components of the PDAC stroma do not eliminate, but rather tolerate tumor cells [34, 35]. Despite its implication for PDAC progression and therapy resistance, therapeutic efforts aiding at stroma depletion have not proved successful in combatting PDAC [28]. Rather, the strong plasticity of the PDAC TME argues for reprogramming the PDAC stroma in favor of a less aggressive and more therapy susceptible TME. Not surprisingly, epigenetic mechanisms play a pivotal role in controlling the dynamic plasticity of the cellular components of the PDAC stroma and hence represent promising targets to interfere with the cellular heterogeneity of the disease.

**Epigenetic Treatment Strategies to Target the PDAC TME**

Several studies have investigated the role of DNA methylation processes in the regulation of gene expression in multiple components of the TME. For instance, blockade of DNA methylation by applying the DNA methyltransferase (DNMT) inhibitor 5-azacytidine reduced PDAC progression by interfering with global DNA methylation in epithelial PDAC cells and cancer-associated fibroblasts (CAFs) (Fig. 2) [36]. Further supporting the utility of DNMT inhibition in PDAC TME reprogramming strategies is the use of BET inhibitors, which have been shown to reduce CAF activity and improve chemotherapy sensitivity in PDAC [37].

**Fig. 2.** Epigenetic targeting strategies of the PDAC TME. The main challenge in utilizing epigenetic therapeutic strategies to tackle the cellular heterogeneity of PDAC is associated with the complex and multifactorial role of epigenetic regulation occurring in the different cellular PDAC compartments. Consequently, inhibition of epigenetic regulators can shift the cellular composition of the TME toward a more or less tumor-promoting stroma composition. The cartoon was created with BioRender.com. PDAC, pancreatic ductal adenocarcinoma; BET, bromodomain and extra-terminal motif; TME, tumor microenvironment; PSC, pancreatic stellate cell; CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage; ICC, innate immune cell components; ECM, extracellular matrix; HDAC, histone deacetylase.
The complexity of epigenetic strategies in general, and in remodeling the pancreatic TME in particular. With regard to targeting HDAC proteins, this complexity is even increased given the number of HDAC protein family members and their respective diverse functional involvements [50]. For instance, HDAC proteins are not only mediating HDAC and hence transcriptional regulation but also interfere with posttranslational acetylation of a plethora of target proteins [51]. Consequently, the global and partially unpredictable effects of the diverse HDAC proteins and, therefore of their inhibition, represent a significant obstacle for HDAC inhibition in PDAC treatment. Hence, and in the interest of higher drug specificities, currently active clinical trials exploring HDAC inhibition in PDAC treatment concentrate on HDAC class I and II specific inhibitors (Table 1).

EZH2 is another chromatin regulatory protein, which directs TME-reprogramming processes in PDAC. Interestingly, and as illustrated in Ezh2-deficient and Kras-mutant transgenic mouse models of PDAC, conditional Ezh2 deficiency resulted in increased accumulation of CD11b+ macrophages, Gr-1+ neutrophils, and CD11c+ dendritic cells in the pancreas, indicating a higher recruitment of innate immune system players (Fig. 2) [52, 53]. These shifts in the immune compartment of the TME were accompanied by increased collagen deposition and aSMA expression and strongly promoted pancreatic carcinogenesis [52]. Given that the ablation of Ezh2 in this model specifically occurred in the epithelial, but not in the stromal cells, these data further imply a strong communication between the epithelial and the TME cellular components of the pancreas. This note is further supported by the fact that the application of the Cox2 inhibitor nimesulide rescued the inflammatory response in these mice and prevented the formation of advanced PDAC precursor lesions [52]. The observations made in the Ezh2-deficient transgenic model are accomplished by several reports, indicating the critical involvement of epigenetic players in controlling immune cell regulation in PDAC development in progression. For instance, epigenetic mechanisms impact on antigen processing and presentation by tumors cells, control the transcription of immune-suppressive cytokines, and impair cytotoxic T-cell function [54]. Accordingly, the CD274 gene, encoding for the immune checkpoint inhibitor PD-L1, is heavily enriched for the H3K4me3 histone mark, rendering its promoter transcriptionally active. This histone mark is installed by the methyltransferase MLL1. Consequently, MLL1 inhibition has been reported to partially prevent increased PD-L1 expression and subsequent immune cell evasion [55]. A similar efficacy in overcoming immune cell evasion in PDAC has been shown upon combining the checkpoint inhibitors anti-CD-1 or anti-CTLA-4 with HDAC inhibition. This combinatorial treatment strategy led to a significant abundance of cytotoxic T cells by decreasing the activity of
Conclusion

Without doubt, epigenetic regulatory proteins represent pivotal drivers of PDAC tumor progression and therapy resistance, not only, but particularly by determining the cellular and molecular heterogeneity of the disease. Translational studies conducted within the last years have convincingly demonstrated the utility of epigenetic targeting in interfering with PDAC plasticity and switching between different cellular and molecular states. Nevertheless, in contrast to other tumor entities, epigenetic targeting of PDAC is still in its infancy. This is also indicated by the relatively low number of epigenetic drugs which qualify for clinical testing in PDAC (Table 1). Despite significant efforts, important technical advances and the increasing understanding of the implications of PDAC heterogeneity for the disease course and outcome, we have only started to dissect the molecular underpinnings and the upstream regulatory components, which finally give rise to the complex epigenetic landscape evident in PDAC. The dynamic nature of epigenetic alterations allows reverting cellular states and conditions in favor of a less aggressive and more therapy susceptible molecular and cellular makeup of PDAC. However, epigenetic mechanisms often play contrary roles in different cellular components of PDAC, with tumor-promoting activities in one, and tumor-suppressive functions in other compartments. Unfortunately, current epigenetic targeting strategies cannot distinguish between the different functional implications of spatially distinct but otherwise identical, epigenetic mechanisms.

In contrast to hematological malignancies in which epigenetic treatment strategies play an increasing role even in monotherapeutic application, it does not seem likely that targeting of 1 epigenetic regulator or even of a family of epigenetic proteins is sufficient to combat PDAC. As described in this article, first promising results have been reported for combinatory treatment strategies, which involve epigenetic targeting. From these studies, we gained first evidence that epigenetic therapies have a strong potential in priming PDAC tumor cells and their TME for additional, predominantly cytotoxic, therapy. Hence, in addition to further disentangle the complex interactions between epigenetic mechanisms and additional regulatory processes evident in PDAC, translational efforts need to unravel the best sequence of epigenetic drug application to combat this dismal disease.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

Funding Sources

This work has been supported by the German Research Foundation (DFG, to E.H.: KFO5002) and the German Cancer Aid (to E.H.: 70114087).

Author Contributions

L.V. wrote the part on epigenetics in PDAC molecular heterogeneity and drafted Figure 1. M.U. wrote the part on PDAC cellular heterogeneity and designed Figure 2 and Table 1. E.H. developed the review’s concept and structure, wrote the introduction and the conclusion, and compiled all sections of this review.

References

1. Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518(7540):495–501.
2. Chan-Seng-Yue M, Kim JC, Wilson GW, Ng K, Figueroa EF, O’Kane GM, et al. Transcription phenotypes of pancreatic cancer are driven by genomic events during tumor evolution. Nat Genet. 2020;52(2):231–40.
3. Bailey P, Chang DK, Nones K, Johns AL, Patch AM, Gingras MC, et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature. 2016;531(7592):47–52.
4. Collisson EA, Sadanandam A, Olson P, Gibb WJ, Truitt M, Gu S, et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011;17(4):500–3.
5. Moffitt RA, Marayati R, Flate EL, Volmar KE, Loeza SG, Hoadley KA, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015;47(10):1168–78.
6. Raphael BJ, Hruban RH, Aguirre AJ, Moffitt RA, Yeh JJ, Stewart C, et al. Integrated genomic characterization of pancreatic ductal adenocarcinoma. Cancer Cell. 2017;32(2):185–203.e13.
7. Puleo F, Nicolle R, Blum Y, Cros J, Marisa L, Demetter P, et al. Stratification of pancreatic ductal adenocarcinomas based on tumor and microenvironment features. Gastroenterology. 2018;155(6):1999–2013.e3.
8. Lomberk G, Blum Y, Nicolle R, Nair A, Godonkar KS, Marisa L, et al. Distinct epigenetic landscapes underlie the pathobiology of pancreatic cancer subtypes. Nat Commun. 2018;9(1):1978.
9. Hessmann E, Johnsen SA, Siveke JT, Ellenrieder V. Epigenetic treatment of pancreatic cancer: is there a therapeutic perspective on the horizon? Gut. 2017;66(1):168–79.
10. Alexandrov LB, Nik-Zainal S, Siu HC, Leung SY, Stratton MR. A mutational signature in gastric cancer suggests therapeutic strategies. Nat Commun. 2015;6:8683.
11. Bettaieb A, Paul C, Plenchette S, Shan J, Choucane I, Ghiringhelli F. Precision medicine in breast cancer: reality or utopia? J Transl Med. 2017;15(1):139.
12. Politi K, Herbst RS. Lung cancer in the era of precision medicine. Clin Cancer Res. 2015;21(10):2213–20.
13 Binkan AY, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature*. 2012; 491(7424):399–405.

14 Aug KL, Fischer SE, Denroche RE, Jang GH, Dedda O, Creighton S, et al. Genomics-driven precision medicine for advanced pancreatic cancer: early results from the COMPASS Trial. *Clin Cancer Res*. 2018;24(6):1344–54.

15 Lomberk G, Dusetti N, Iovanna J, Urrutia R. Emerging epigenomic landscapes of pancreatic cancer in the era of precision medicine. *Nat Commun*. 2019;10(1):3875.

16 Pott S, Lieb JD. What are super-enhancers? *Nat Genet*. 2015;47(1):8–12.

17 Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, et al. Master transcription factors and mediator stabilize super-enhancers at key cell identity genes. *Cell*. 2013;153(2):307–19.

18 Hamdan FH, Johnsen SA. DeltaN63-dependent super-enhancers define molecular identity in pancreatic cancer by an interconnected transcription factor network. *Proc Natl Acad Sci U S A*. 2018;115(52):E12343–52.

19 Somervile TDD, Xu X, Miyabayashi K, Tiraic H, Clayley CR, Maia-Silva D, et al. TP63-mediated enhancer reprogramming drives the squamous subtype of pancreatic ductal adenocarcinoma. *Cell Rep*. 2018;25(7):1741–55.e7.

20 Andricovich J, Perkall S, Kai Y, Casasanta N, Somerville TDD, Xu Y, Miyabayashi K, Tiriac H, Hui CC, Angers S. Gli proteins in development:  early results from the COMPASS Trident super enhancers define molecular identity factors and mediator establish super-enhancer sensitivity to BET inhibitors. *Cancer Cell*. 2018;33(3):512–26.e8.

21 Patil S, Steuber B, Kopp W, Kari Y, Urbach L, Patzak MS, Klein L, et al. Hypomethylating therapy in pancreatic cancer: unmasking new vulnerabilities and the execution of an intrinsic ifn program showing hypomethylation of repetitive elements in human pancreatic cancer cells. *Clin Exp Metastasis*. 2016;33(3):225–30.

22 Espinet E, Gu Z, Imbusch CD, Giese NA, Zhong S, Painchaud J, et al. Depletion of macrophages increases thera-peutic response to gemcitabine in murine pancreatic cancer. *Cancers*. 2020;12(7):1978.

23 Neesse A, Bauer CA, Ohland D, Lath M, Buchholz M, Michl P, et al. Stromal biology and therapy in pancreatic cancer: ready for clinical translation? *Gut*. 2019;68(1):159–71.

24 Guo S, Contratto M, Miller G, Leichman L, Wu J. Immunotherapy in pancreatic cancer: unleashing its potential through novel combinations. *World J Clin Oncol*. 2017;8(3):230–40.

25 Shyka R, Gonda T, Quante M, Salas M, Kim S, Brooks J, et al. Histone deacetylase inhibitors provoke a tumor supportive phenotype in pancreatic cancer associated fibroblasts. *Oncotarget*. 2017;8(12):19074–88.

26 Neesse A, Kari V, Ramu I, et al. Fibroblast drug scavenging increases intratumoral gemcitabine accumulation in murine pancreas cancer. *Gut*. 2018;67(1):95–707.

27 Buchholz SM, Goetze RG, Singh SK, Ammer-Hermneau C, Richards FM, Jodrell DJ, et al. Depletion of macrophages increases therapeutic response to gemcitabine in murine pancreatic cancer. *Cancers*. 2020;12(7):1978.

28 Neesse A, Bauer CA, Ohland D, Lath M, Buchholz M, Michl P, et al. Stromal biology and therapy in pancreatic cancer: ready for clinical translation? *Gut*. 2019;68(1):159–71.

29 Hui CC, Angers S. Gli proteins in development and disease. *Annu Rev Cell Dev Biol*. 2011;27:513–37.

30 Adams CR, Htwe HH, Marsh T, Wang AL, Montoya ML, Subbaraj L, et al. Transcriptional control of subtype switching ensures adaptation and growth of pancreatic cancer. *Cell*. 2019;178(4):5313.

31 Daemen A, Peterson D, Sahu N, McCord R, Du X, Liu B, et al. Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors. *Proc Natl Acad Sci U S A*. 2015;112(32):E4410–7.

32 Brunton H, Caligiuri G, Cunningham R, Upstill-Goddard R, Bailey UM, Garnier IM, et al. HNF4A and GATA6 define molecular identity of pancreatic cancer cells. *Cell Rep*. 2020;31(6):13121–26.

33 Garcia PL, Miller AL, Kreitzburg KM, Council LN, Gamblin TL, Christein JD, et al. The BET bromodomain inhibitor JQ1 suppresses growth of pancreatic ductal adenocarcinoma in patient-derived xenograft models. *Oncogene*. 2016;35(7):833–45.

34 Sahai V, Kumar K, Knab LM, Chow CR, Raza SS, Bentrem DJ, et al. BET bromodomain inhibitors block growth of pancreatic cancer cells in three-dimensional collagen. *Mol Cancer Ther*. 2014;13(7):1907–17.

35 Yamamoto K, Tateishi K, Kudo Y, Hoshikawa M, Tanaka M, Nakatsuka T, et al. Stromal remodeling by the BET bromodomain inhibitor JQ1 suppresses the progression of human pancreatic cancer. *Oncotarget*. 2016;7(38):56469–84.

36 Filippakopoulos P, Knapp S. Targeting bromodomain: epigenetic readers of lysine acetylation. *Nat Rev Drug Discov*. 2014;13(5):337–56.

37 Filippakopoulos P, Qi J, Picaid S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomain. *Nature*. 2010;468(7327):1067–73.

38 Kumar K, DeCant BT, Grippo PJ, Hwang RF, Bentrem DJ, Ebine K, et al. BET inhibitors block pancreatic stellate cell collagen I production and attenuate fibrosis in vivo. *JCI Insight*. 2017;2(3):e88032.

39 Baretzi M, Ahuja N, Azad NS. Targeting the epigenome of pancreatic cancer for therapy: challenges and opportunities. *Ann Pancreatic Cancer*. 2019;2.

40 Nguyen AH, Elliott IA, Wu N, Matsumura C, Vogelauer M, Attar N, et al. Histone deacetylase inhibitors provoke a tumor supportive phenotype in pancreatic cancer associated fibroblasts. *Oncotarget*. 2017;8(12):19074–88.

41 Schneider G, Kramer OH, Fritsche P, Schuler S, Schmid RM, Saur D. Targeting histone deacetylases in pancreatic ductal adenocarci-noma. *J Cell Mol Med*. 2010;14(6a):1255–63.

42 Kulka LAM, Fangmann PV, Panfilova D, Olszcha H. Impact of HDAC inhibitors on protein quality control systems: consequences for precision medicine in malignant disease. *Front Cell Dev Biol*. 2020;8(425):425.

43 Mallen-St Clair J, Soydamer-Aze loglu R, Lee KE, Taylor L, Livanos A, Pylaye-Gupta Y, et al. EZH2 couples pancreatic regeneration to neoplastic progression. *Genes Dev*. 2012;26(3):439–44.

44 Chen NM, Nesse A, Dyck ML, Steuber B, Koenig AO, Lubesdes-Martelato C, et al. Context-dependent epigenetic regulation of nuclear factor of activated T cells 1 in pancreatic plasticity. *Gastroenterology*. 2017;152(6):1507–20.e15.

45 Heninger E, Krueger TE, Lang JM. Augmenting antitumor immune responses with epigenetic modifying agents. *Front Immunol*. 2015;6:29.

46 Lu C, Paschall AV, Shi H, Savage N, Waller JL, Sabbatini ME, et al. The MLL1-H3K4me3 axis mediates PD-L1 expression and pancreatic cancer immune evasion. *J Cancer Invest*. 2017;10(96):djw283.

47 Christmas BJ, Rafie CI, Hopkins AC, Scott BA, Ma HS, Cruz KA, et al. Entinostat converts immune-resistant breast and pancreatic cancers into checkpoint-responsive tumors by reprogramming tumor-infiltrating MDCSs. *Cancer Immunol Res*. 2018;6(12):1561–77.

48 Gerrard DL, Boyd JR, Stein GS, Jin VX, Fritze S. Disruption of broad epigenetic domains in PDAC cells by HAT inhibitors. *Epigenomes*. 2019;3(2).