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# Whole-Genome Mapping of Small Molecule Targets for Cancer Medicine

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## Abstract

Cancers display intra- and intertumoral heterogeneity, which poses challenges to small molecule intervention. Studying drug responses on a whole-genome and transcriptome level using next generation sequencing (NGS) has revolutionized our understanding of how small molecules intervene in cells, which helps study and potentially predict treatment outcomes. Some small molecules act directly at the genomic level by targeting DNA or chromatin proteins. Here, we review recent advances in establishing whole-genome and transcriptome maps of small-molecule targets, comprising chromatin components or downstream events. We also describe recent advances in studying drug responses using single-cell RNA and DNA sequencing. Furthermore, we discuss how this fundamental research can be taken forward to devise innovative personalized treatment modalities.

## Introduction

Since the advent of NGS [1], this technology has been used extensively, ranging from fundamental cell biology studies to sequencing patient cohorts. This has allowed to monitor the cellular effects of small molecules on a genomic and transcriptomic level. Several anti-cancer drugs, including the commonly used cisplatin and nitrosoureas, can directly interact with chromatin. DNA sequencing of treated cells can deliver valuable information on their mechanisms of action, treatment resistance and potentially devise therapeutic alternatives [2]. The development of orthogonal approaches to map genomic sites of small molecules using Chem-seq holds great promise to unravel the complex genetic and epigenetic drug response in diseases, including cancers [2-5]. This information is invaluable, especially given the often

pleiotropic nature of small molecules in cells [6]. Direct DNA and RNA-sequencing of tumors can also give insights to drug responses on a more general scale and in particular transcriptome analyses have helped profile tumors and resistant phenotypes [7]. In recent years, the advent of single-cell (sc) sequencing including scRNA-seq and scDNA-seq has helped profile tumor evolution before and after drug treatment. Here, we discuss how recent studies that used these approaches have shed light on the complexity of cancer heterogeneity in light of small-molecule intervention and drug treatment, and how this information could be used to devise appropriate treatment modalities for patients in the future.

## **Small molecule genome mapping**

### **Whole-genome and transcriptome maps of DNA-interacting small molecules**

The earliest studies looking at establishing genomic maps of small molecules interacting with DNA involved G-quadruplex-(G4) interacting small molecules [8]. G4 are non-Watson-Crick nucleic acid topologies linked to diverse biological processes, including telomere maintenance, transcription, replication and genome rearrangements [8-10]. The extensive aromatic surface of G4 allows for specific small molecule interactions to alter biological processes, which require DNA templates. This was exemplified in early studies attempting to specifically target promoter G4 in genes such as the *MYC* oncogene to interfere with gene expression [11]. However, the complexity of gene regulatory networks, and the lack of whole-genome data of the effect of small molecules at the time, challenged this hypothesis. The development of the small molecule pyridostatin (PDS), highly selective for G4 [12], formed the basis for the development of affinity-based isolation of G4 from cells. An early approach using PDS with a biotin tag to isolate G4 from cells (Fig 1a) [13] showed how a small molecule could be used to establish its whole-genome interaction maps with chromatin from cell lysates. However, the dynamic nature of G4 made target isolation from cell lysates moderately representative of native conditions in living cells and importantly, biotin tagging altered the potency of PDS. Bulky biotinylated polyethylene glycol linkers may also adversely affect cellular uptake, metabolism and small-molecule–target interactions, thereby offering limited value in the context of identifying small-molecule target sites. Thus, this approach was developed further to introduce biorthogonal groups to PDS, such as an alkyne, to allow experiments in living cells with little effect on the biological properties of the molecule. The use of click chemistry on PDS - a strategy now widely used to identify small molecule targets in cells [14] - was exploited to introduce a fluorophore onto the molecule (Fig 1b) [15]. This strategy identified overlapping genomic binding domains of PDS and the human G4 unwinding helicase PIF1 $\alpha$ , a protein previously described to unwind G4 structures [16]. Importantly, click chemistry on a PDS-

derivative identified these overlapping sites on cells fixed prior to addition of the small molecule, which indicated that G4 structures exist prior to drug addition [15]. This work has further been substantiated by a recent report of a crystal structure of the helicase DHX36 with a G4 [17]. Taken together, this provided strong evidence for the existence of G4 structures in human cells. In addition, double-strand DNA breaks induced by PDS [12] were used to identify affected genomic sites using immunoprecipitation of genomic DNA bound to the DNA-damage marker phosphorylated histone H2AX ( $\gamma$ H2AX), followed by NGS (ChIP-seq) (Fig 1b). This was a pioneering approach for mapping functional sites of action of a small molecule genome-wide [15]. This approach identified novel G4-containing genomic sites and genes, susceptible to be targeted by a G4-interacting small molecule. Interestingly, G4 density predicted by means of bioinformatics did not always correlate with functional sites identified experimentally, indicating that other factors, such as chromatin conformation, influences drug targeting in cells. This study prompted a similar approach to provide a genome-wide map of G4, using PDS or stabilizing ions such as  $K^+$  to induce G4 structures that can stall the polymerase during the elongation step in the sequencing process [18] (Fig 1c). These polymerase stalling sites were then used to deliver a map of possible G4 structures susceptible to be folded during replication. Although this approach relied on G4 forming sequences, which not necessarily cause DNA damage, the major caveat here was the use of naked DNA, excluding other chromatin factors that play a role during replication. The same approach was used later to map G4 in RNA [19]. RNA G4 maps were also established using another small molecule probe labelled BioTASQ, which is essentially an artificial G-tetrad comprised of guanines fused with a linker to a cyclen scaffold [20]. This molecule was employed to fish out G4 forming sequences to establish a G4 RNA map. Another approach to establish G4 whole-genome maps employed a G4-specific antibody [21] to pull-down G4 forming sequences [22,23] (Fig 1d). This approach identified ~10,000 G4 structures in human chromatin, predominantly in regulatory, nucleosome-depleted regions. Interestingly, G4 formation was associated with increased transcriptional activity, as shown by HDAC inhibitor-induced chromatin relaxation. Although G4 interacting small molecules have not yet made it into the clinic, these pioneering approaches could be applied to other chromatin-interacting chemotherapeutic agents, mapping their interaction sites to gain mechanistic insight and to compare the resulting maps between tumors of different patients. Indeed, a click chemistry approach to pull-down interacting genomic sites of a cisplatin analog was attempted, which revealed that chromatin is an important factor for drug accessibility [24]. This could now be taken forward to study chromatin effects on cisplatin treatment, especially in light of acquired resistance to this class of drugs.

## Mapping drug targets using Chem-seq

Early work on biotinylated G4-binding small molecules inspired other approaches using pull-down strategies of chromatin-interacting small molecules with biotin affinity tags (Fig 2a). JQ1 is a small molecule that specifically interacts with BET bromodomains of proteins [25]. A landmark study employed a biotinylated version to identify genome-wide binding sites of bromodomain-containing proteins, notably the so-called bromodomain (BRD)-containing family of proteins [3]. To this end, the authors pulled down chromatin bound to biotinylated JQ1 and then subjected bound DNA fragments to NGS. Sequencing revealed that biotinylated JQ1 isolated DNA fragments of the same regions pulled down by ChIP-seq experiments using specific antibodies against the BRD2, BRD3 and BRD4 proteins (Fig 2b). Essentially orthogonal to the earlier work on G4, this work demonstrated that a biotinylated small molecule coupled to NGS could be used to map its site of interaction with chromatin, a process the authors coined Chem-seq.

A similar Chem-seq approach was used to determine the genomic targeting of the small molecule SD70, a drug that inhibits dihydrotestosterone (DHT)-induced androgen receptor-mediated gene translocations in prostate cancer [4]. The authors employed a biotinylated derivative of SD70 to pull-down DNA, and sequencing revealed colocalization of the small molecule with androgen receptor-bound regulatory enhancers. This work revealed that the small molecule did not target androgen receptors directly, but rather inhibited the lysine demethylase activity of KDM4C, a known mediator of androgen receptor transcriptional regulation. Thus, Chem-seq allowed to identify the target of this small molecule and to elucidate its molecular mode of action in cells. This approach is therefore a powerful tool that could be used to delineate complex drug responses of chromatin-interacting small molecules, potentially acting differentially between patients.

In a recent study, using the alkyne substituted JQ1-derivative (JQ1-PA) or the strained alkene-based JQ1-TCO, click chemistry-based proteomics and sequencing (Click-seq) allowed to explore the gene regulatory function of BRD4 and the transcriptional changes induced by BET inhibitors [5] (Fig 2c). JQ1-PA and JQ1-TCO have very small tags and exhibited properties very close to the parent compound, an important advantage over biotinylated JQ1-analogues. These molecules were used to perform click chemistry using specific fluorophores *in cellulo* in different tissues, which highlighted the heterogeneity of drug activity within tumor cells located throughout a diseased tissue. This approach demonstrated that both NGS and small molecule imaging could potentially be used together for a preclinical assessment of drug activity, and this strategy could be employed to tailor drug treatment in a personalized medicine approach in oncology [2].

## **Mapping drug responses in the genome**

This topic is vast and there have been some key recent advances using NGS to profile drug responses in cancer. Small molecules acting on epigenetic regulators such as histone methyltransferases have attracted attention of the scientific community, in particular for the development of compounds that can be exploited to assert an effect on the epigenetic landscape of cancer. The small molecule GSK126, a selective inhibitor of EZH2 methyltransferase activity, involved in repressing gene expression through methylation of histone H3 on lysine 27, proved to be a valuable tool to assess the response of GSK126-treated cells using a ChIP-seq approach against this histone mark [26]. The authors showed using GSK126 that pharmacological inhibition of EZH2 activity may provide a promising treatment for EZH2 mutant lymphoma, highlighting how NGS of the drug response against a chromatin modifying small molecule can give mechanistic and potentially therapeutic insights. On a wider scale, NGS has helped profile drug responses in an array of different cancer tissues. This has sparked the creation of big repositories of data, which could help design data banks to design specialized personalized anti-cancer treatments [27]. Furthermore, these approaches have been used in clinical studies, showing that genomic and transcriptomic profiling can be useful to predict treatment outcomes and to suggest improvements in therapy [28,29]. Innovative approaches have exploited automated high-throughput workflows for RNA-seq that take advantage of barcoding individual mRNA sequences, which are then subjected to repeated PCR amplification. This approach, coined DRUG-seq, can generate transcription profiles of high fidelity and throughput, and in one study, 433 compounds across 8 treatment doses were profiled [30]. In another study, investigating 5-FU, a drug commonly used for several solid tumors, NGS revealed that prolonged treatment of organoids with 5-FU interferes with nucleotide synthesis and incorporates into DNA, which causes mutations. This signature was found in patient tumors, rationalizing how drug resistance could occur, and how prolonged 5-FU treatment can cause secondary malignancies [31]. Taken together, this work shows how indispensable NGS has become to profile drug responses in patients. It also holds the premise to be instrumental in devising personalized chemotherapeutic treatments.

## **Single-cell analyses of drug responses in cancer**

NGS has provided the means to decipher genomic and transcriptomic intratumoral heterogeneity of cancers [32-34] (Fig 3a). In recent years, with the development of single-cell sequencing approaches, important information about intratumoral cell heterogeneity has been gathered, especially with respect to drug-resistant tumor subpopulations. The complexity of cancer heterogeneity is often further increased by the presence of cancer-associated cells (for

example fibroblasts [35] or macrophages [36]), which can make up a large part of the tumor and/or its microenvironment (Fig 3a). Important recent reports using single-cell RNA-seq (scRNA-seq) revealed that the acquisition of malignant phenotypes after treatment resulted from the selection of treatment-resistant cells that were already present in the initial tumor [37,38]. This included enhanced mesenchymal and growth factor signaling, typical signatures of an epithelial-to-mesenchymal (EMT) transition [37]. Interestingly, these findings argue that eradicating cancer stem cells or persister cancer cells should be a prime objective for the development of the next generation of anti-cancer therapies [39]. This could be achieved by targeting this subpopulation of cells using novel approaches in line with their distinct biochemistry and epigenetic signatures, such as their addiction to iron and copper [40,41]. Using breast cancer models, it was demonstrated that taxol-resistant cells exhibit specific RNA expression profiles involved in microtubule organization and stabilization [42]. Another study on renal cell carcinoma and their metastatic sites using scRNA-seq showed that the primary tumor and metastases responded differentially to treatment with tyrosine kinase inhibitors, commonly used to treat this type of cancer [43]. Indeed, the authors demonstrated that a co-treatment with the tyrosine inhibitors afatinib and dasatinib improved treatment outcome in some tumors, suggesting that this approach can help fine tune anti-cancer treatments in a personalized manner. Interesting data on the analyses of malignant cell states that promote immune evasion showed a signature for immunotherapy resistance in melanoma, which could be used to devise appropriate treatments [44]. Using scRNA-seq on human myeloma [45] and melanoma [46], expression profiles were devised to predict treatment outcome by coupling this to predictive algorithms, the so-called machine-learning approach.

More challenging has been epigenetic profiling using ChIP-seq at the single-cell level. A landmark study showed that a subset of cells within untreated drug-sensitive breast tumors share a common chromatin signature with resistant cells, undetectable using bulk approaches [47]. This approach allowed identification of cell subpopulations based on epigenetic profiles using histone marks (Fig 3b). Transposase-accessible chromatin sequencing in single cells (scATAC-seq) of basal cell carcinoma and intratumoral T-cells revealed regulatory programs and chromatin regulators, and therapy-resistant cells in the bulk population [48] (Fig 3c). Although these single-cell DNA sequencing approaches harbor interesting premises, single-cell ChIP-seq is still in its infancy and at a too early stage to be exploited routinely for therapeutic advancements, something that may well change in the near future.

## Conclusions and Perspectives

NGS has provided unprecedented means to understand the genetic and epigenetic landscapes of cancer. Approaches like Chem-seq have provided mechanistic insights into chromatin-targeting small molecules, a powerful technique especially in conjunction with *in cellulo* imaging of these compounds [14]. Whole genome sequencing and transcriptomics analyses of drug responses have further refined the understanding of chemotherapy treatments and provided the means to predict or unravel treatment outcomes in some cases. In recent years, single-cell sequencing has given the means to study intratumoral heterogeneity with unprecedented resolution, and how distinct cell subpopulations react to treatment.

A common theme that has emerged from recent studies, is that failure in cancer chemotherapy almost exclusively underlies gain of treatment resistance arising from a subpopulation of tumor cells. While this notion is hardly novel, NGS has provided unprecedented means to characterize these cell populations. New effort should now be channeled as to how these cells could be eradicated, which could help prevent relapse and metastases formation in various cancers. In addition, cohort studies have identified intratumoral heterogeneity, and these approaches can now be used to assess therapeutic responses to propose alternatives in a personalized medicine approach, and potentially to predict treatment outcomes to improve anticancer regimens.

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## References

1. Furey WS, Joyce CM, Osborne MA, Klenerman D, Peliska JA, Balasubramanian S: **Use of fluorescence resonance energy transfer to investigate the conformation of DNA substrates bound to the Klenow fragment.** *Biochemistry* 1998, 37:2979-2990.
2. Rodriguez R, Miller KM: **Unravelling the genomic targets of small molecules using high-throughput sequencing.** *Nat. Rev. Genet.* 2014, 15:783-796.
3. Anders L, Guenther MG, Qi J, Fan ZP, Marineau JJ, Rahl PB, Loven J, Sigova AA, Smith WB, Lee TI, et al.: **Genome-wide localization of small molecules.** *Nat. Biotechnol.* 2014, 32:92-96.
4. Jin C, Yang L, Xie M, Lin C, Merkurjev D, Yang JC, Tanasa B, Oh S, Zhang J, Ohgi KA, et al.: **Chem-seq permits identification of genomic targets of drugs against androgen receptor regulation selected by functional phenotypic screens.** *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111:9235-9240.

- \*\*5. Tyler DS, Vappiani J, Caneque T, Lam EYN, Ward A, Gilan O, Chan YC, Hienzsch A, Rutkowska A, Werner T, et al.: **Click chemistry enables preclinical evaluation of targeted epigenetic therapies.** *Science* 2017, 356:1397-1401.

A study employing Chem-seq and ChiP-seq combined with small molecule imaging using click chemistry in cells and mouse tissues. This study provides a framework for a potential preclinical assessment of drug treatment.

6. Müller S: **DNA Damage-inducing Compounds: Unraveling their Pleiotropic Effects Using High Throughput Sequencing.** *Curr. Med. Chem.* 2017, 24:1558-1585.
7. Morash M, Mitchell H, Beltran H, Elemento O, Pathak J: **The Role of Next-Generation Sequencing in Precision Medicine: A Review of Outcomes in Oncology.** *J. Pers. Med.* 2018, 8.
8. Müller S, Rodriguez R: **G-quadruplex interacting small molecules and drugs: from bench toward bedside.** *Expert Rev. Clin. Pharmacol.* 2014, 7:663-679.
9. Bochman ML, Paeschke K, Zakian VA: **DNA secondary structures: stability and function of G-quadruplex structures.** *Nat. Rev. Genet.* 2012, 13:770-780.
10. Rhodes D, Lipps HJ: **G-quadruplexes and their regulatory roles in biology.** *Nucleic Acids Res.* 2015, 43:8627-8637.
11. Siddiqui-Jain A, Grand CL, Bearss DJ, Hurley LH: **Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription.** *Proc. Natl. Acad. Sci. U.S.A.* 2002, 99:11593-11598.
12. Rodriguez R, Müller S, Yeoman JA, Trentesaux C, Riou JF, Balasubramanian S: **A novel small molecule that alters shelterin integrity and triggers a DNA-damage response at telomeres.** *J. Am. Chem. Soc.* 2008, 130:15758-15759.
13. Müller S, Kumari S, Rodriguez R, Balasubramanian S: **Small-molecule-mediated G-quadruplex isolation from human cells.** *Nat. Chem.* 2010, 2:1095-1098.
- \*\*14. Cañeque T, Müller S, Rodriguez R: **Visualizing biologically active small molecules in cells using click chemistry.** *Nat. Rev. Chem.* 2018, 2:202–215.

This is a comprehensive review of small-molecule tagging and visualization in cells, an approach that gives invaluable information for the mechanistic action of small molecules.

- \*\*15. Rodriguez R, Miller KM, Forment JV, Bradshaw CR, Nikan M, Britton S, Oelschlaegel T, Xhemalce B, Balasubramanian S, Jackson SP: **Small-molecule-induced DNA damage identifies alternative DNA structures in human genes.** *Nat. Chem. Biol.* 2012, 8:301-310.

This is a key paper mapping small molecule-induced DNA damage sites throughout the genome using ChIP-seq against a DNA damage marker. It also shows first important visual evidence of G4 formation in cells using click chemistry and staining against the human helicase hPIF1 $\alpha$ .

16. Sanders CM: **Human Pif1 helicase is a G-quadruplex DNA-binding protein with G-quadruplex DNA-unwinding activity.** *Biochem J.* 2010, 430:119-128.
17. Chen MC, Tippana R, Demeshkina NA, Murat P, Balasubramanian S, Myong S, Ferre-D'Amare AR: **Structural basis of G-quadruplex unfolding by the DEAH/RHA helicase DHX36.** *Nature* 2018, 558:465-469.
18. Chambers VS, Marsico G, Boutell JM, Di Antonio M, Smith GP, Balasubramanian S: **High-throughput sequencing of DNA G-quadruplex structures in the human genome.** *Nat. Biotechnol.* 2015, 33:877-881.

19. Kwok CK, Marsico G, Sahakyan AB, Chambers VS, Balasubramanian S: **rG4-seq reveals widespread formation of G-quadruplex structures in the human transcriptome.** *Nat. Methods* 2016, 13:841-844.
20. Yang SY, Lejault P, Chevrier S, Boidot R, Robertson AG, Wong JMY, Monchard D: **Transcriptome-wide identification of transient RNA G-quadruplexes in human cells.** *Nat. Commun.* 2018, 9:4730.
21. Biffi G, Tannahill D, McCafferty J, Balasubramanian S: **Quantitative visualization of DNA G-quadruplex structures in human cells.** *Nat. Chem.* 2013, 5:182-186.
- \*\*22. Hänsel-Hertsch R, Beraldi D, Lensing SV, Marsico G, Zyner K, Parry A, Di Antonio M, Pike J, Kimura H, Narita M, et al.: **G-quadruplex structures mark human regulatory chromatin.** *Nat. Genet.* 2016, 48:1267-1272.

This study established a map of G4 in the human genome using a G-quadruplex specific antibody.

23. Hänsel-Hertsch R, Spiegel J, Marsico G, Tannahill D, Balasubramanian S: **Genome-wide mapping of endogenous G-quadruplex DNA structures by chromatin immunoprecipitation and high-throughput sequencing.** *Nat. Protoc.* 2018, 13:551-564.
24. Zacharioudakis E, Agarwal P, Bartoli A, Abell N, Kunalingam L, Bergoglio V, Xhemalce B, Miller KM, Rodriguez R: **Chromatin regulates genome targeting with cisplatin.** *Angew. Chem. Int. Ed.* 2017, 58:6483-6487.
25. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, et al.: **Selective inhibition of BET bromodomains.** *Nature* 2010, 468:1067-1073.
26. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, Liu Y, Graves AP, Della Pietra A, 3rd, Diaz E, et al.: **EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations.** *Nature* 2012, 492:108-112.
27. Gupta S, Chaudhary K, Kumar R, Gautam A, Nanda JS, Dhanda SK, Brahmachari SK, Raghava GP: **Prioritization of anticancer drugs against a cancer using genomic features of cancer cells: A step towards personalized medicine.** *Sci Rep* 2016, 6:23857.
28. Agarwal R, Cao Y, Hoffmeier K, Krezdorn N, Jost L, Meisel AR, Jungling R, Dituri F, Mancarella S, Rotter B, et al.: **Precision medicine for hepatocellular carcinoma using molecular pattern diagnostics: results from a preclinical pilot study.** *Cell Death Dis.* 2017, 8:e2867.
- \*\*29. Rodon J, Soria JC, Berger R, Miller WH, Rubin E, Kugel A, Tsimberidou A, Saintigny P, Ackerstein A, Brana I, et al.: **Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial.** *Nat. Med.* 2019, 25:751-758.

important study of an extensive clinical trial using transcriptomic and genomic profiling to look at drug responses in a cohort.

30. Ye C, Ho DJ, Neri M, Yang C, Kulkarni T, Randhawa R, Henault M, Mostacci N, Farmer P, Renner S, et al.: **DRUG-seq for miniaturized high-throughput transcriptome profiling in drug discovery.** *Nat. Commun.* 2018, 9:4307.
31. Christensen S, Van der Roest B, Besselink N, Janssen R, Boymans S, Martens JWM, Yaspo ML, Priestley P, Kuijk E, Cuppen E, et al.: **5-Fluorouracil treatment induces characteristic T>G mutations in human cancer.** *Nat. Commun.* 2019, 10:4571.
32. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, et al.: **The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups.** *Nature* 2012, 486:346-352.

33. Zhang J, Spath SS, Marjani SL, Zhang W, Pan X: **Characterization of cancer genomic heterogeneity by next-generation sequencing advances precision medicine in cancer treatment.** *Precis. Clin. Med.* 2018, 1:29-48.
34. Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation.** *Cell* 2011, 144:646-674.
35. Kalluri R: **The biology and function of fibroblasts in cancer.** *Nat. Rev. Cancer* 2016, 16:582-598.
36. Cassetta L, Pollard JW: **Targeting macrophages: therapeutic approaches in cancer.** *Nat. Rev. Drug Discov.* 2018, 17:887-904.
37. Brady SW, McQuerry JA, Qiao Y, Piccolo SR, Shrestha G, Jenkins DF, Layer RM, Pedersen BS, Miller RH, Esch A, et al.: **Combating subclonal evolution of resistant cancer phenotypes.** *Nat. Commun.* 2017, 8:1231.
38. Sharma A, Cao EY, Kumar V, Zhang X, Leong HS, Wong AML, Ramakrishnan N, Hakimullah M, Teo HMV, Chong FT, et al.: **Longitudinal single-cell RNA sequencing of patient-derived primary cells reveals drug-induced infidelity in stem cell hierarchy.** *Nat. Commun.* 2018, 9:4931.
- \*\*39. Müller S, Cañeque T, Acevedo V, Rodriguez R: **Targeting Cancer Stem Cells with Small Molecules.** *Isr. J. Chem.* 2017, 57:1-13.

Paper identifying copper as a drugable target of cancer stem cells. This paper also describes the unprecedented visual staining of metformin in mitochondria and describes a unifying mechanism of action of this drug.

- \*\*40. Mai TT, Hamai A, Hienzsch A, Caneque T, Muller S, Wicinski J, Cabaud O, Leroy C, David A, Acevedo V, et al.: **Salinomycin kills cancer stem cells by sequestering iron in lysosomes.** *Nat. Chem.* 2017, 9:1025-1033.

This study identified the mechanism of action of the natural product Salinomycin in selectively killing cancer stem cells. This study also showed that cancer stem cells are addicted to iron, which can be exploited therapeutically.

41. Müller S, Versini A, Sindikubwabo F, Belthier G, Niyomchon S, Pannequin J, Grimaud L, Caneque T, Rodriguez R: **Metformin reveals a mitochondrial copper addiction of mesenchymal cancer cells.** *PLoS One* 2018, 13:e0206764.
42. Lee MC, Lopez-Diaz FJ, Khan SY, Tariq MA, Dayn Y, Vaske CJ, Radenbaugh AJ, Kim HJ, Emerson BM, Pourmand N: **Single-cell analyses of transcriptional heterogeneity during drug tolerance transition in cancer cells by RNA sequencing.** *Proc. Natl. Acad. Sci. U.S.A.* 2014, 111:E4726-4735.
43. Kim KT, Lee HW, Lee HO, Song HJ, Jeong da E, Shin S, Kim H, Shin Y, Nam DH, Jeong BC, et al.: **Application of single-cell RNA sequencing in optimizing a combinatorial therapeutic strategy in metastatic renal cell carcinoma.** *Genome Biol.* 2016, 17:80.
44. Jerby-Arnon L, Shah P, Cuoco MS, Rodman C, Su MJ, Melms JC, Leeson R, Kanodia A, Mei S, Lin JR, et al.: **A Cancer Cell Program Promotes T Cell Exclusion and Resistance to Checkpoint Blockade.** *Cell* 2018, 175:984-997.
45. Mitra AK, Mukherjee UK, Harding T, Jang JS, Stessman H, Li Y, Abyzov A, Jen J, Kumar S, Rajkumar V, et al.: **Single-cell analysis of targeted transcriptome predicts drug sensitivity of single cells within human myeloma tumors.** *Leukemia* 2016, 30:1094-1102.
46. Ho YJ, Anaparthi N, Molik D, Mathew G, Aicher T, Patel A, Hicks J, Hammell MG: **Single-cell RNA-seq analysis identifies markers of resistance to targeted BRAF inhibitors in melanoma cell populations.** *Genome Res.* 2018, 28:1353-1363.

\*\*47. Grosselin K, Durand A, Marsolier J, Poitou A, Marangoni E, Nemati F, Dahmani A, Lameiras S, Reyal F, Frenoy O, et al.: **High-throughput single-cell ChIP-seq identifies heterogeneity of chromatin states in breast cancer.** *Nat. Genet.* 2019, 51:1060-1066.

Key paper using scChIP-seq to identify subpopulations in tumors with epigenetic profiling. This study also describes a novel workflow for scChIP-seq approaches.

\*\*48. Satpathy AT, Granja JM, Yost KE, Qi Y, Meschi F, McDermott GP, Olsen BN, Mumbach MR, Pierce SE, Corces MR, et al.: **Massively parallel single-cell chromatin landscapes of human immune cell development and intratumoral T cell exhaustion.** *Nat. Biotechnol.* 2019, 37:925-936.

Key paper using scATAC-seq to identify subpopulations in the tumor microenvironment.

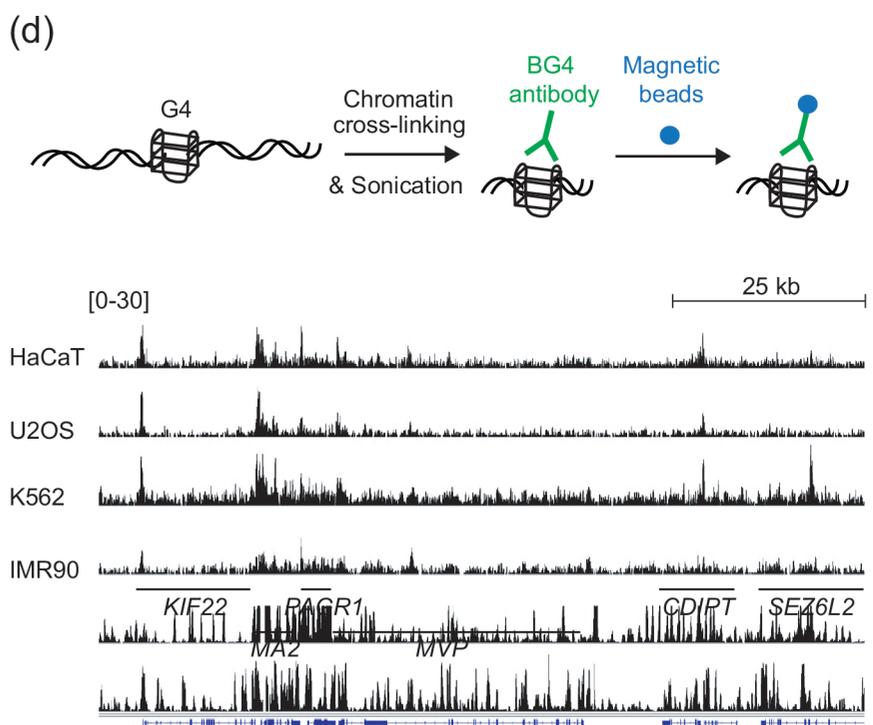
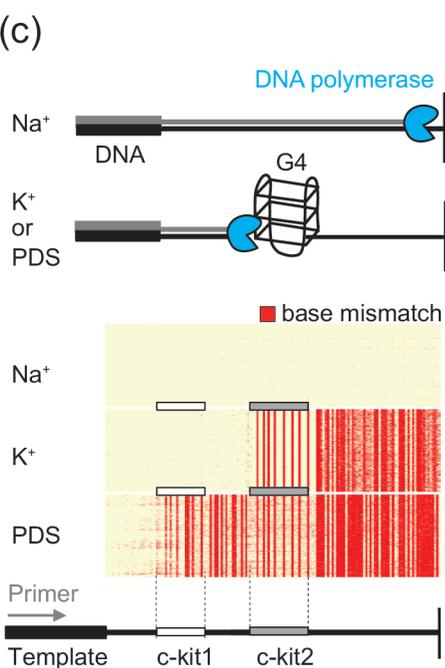
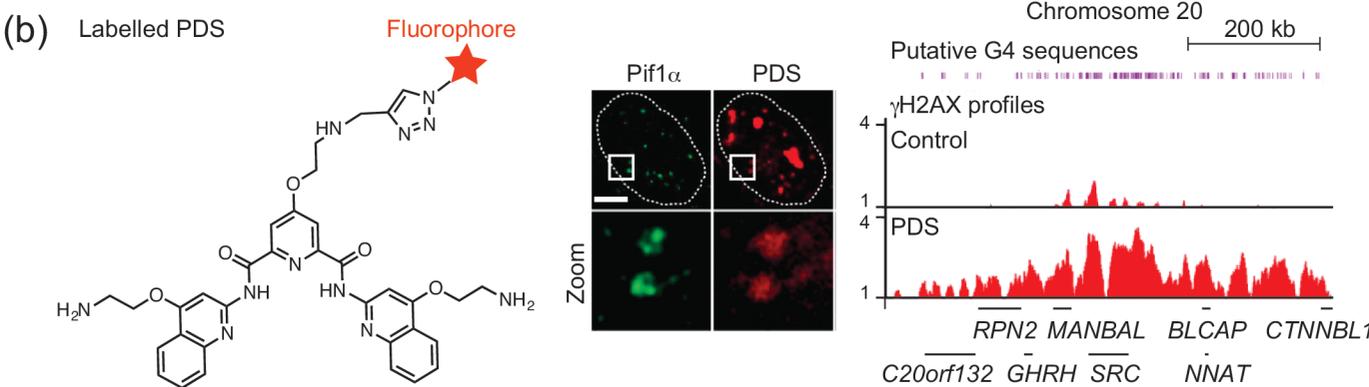
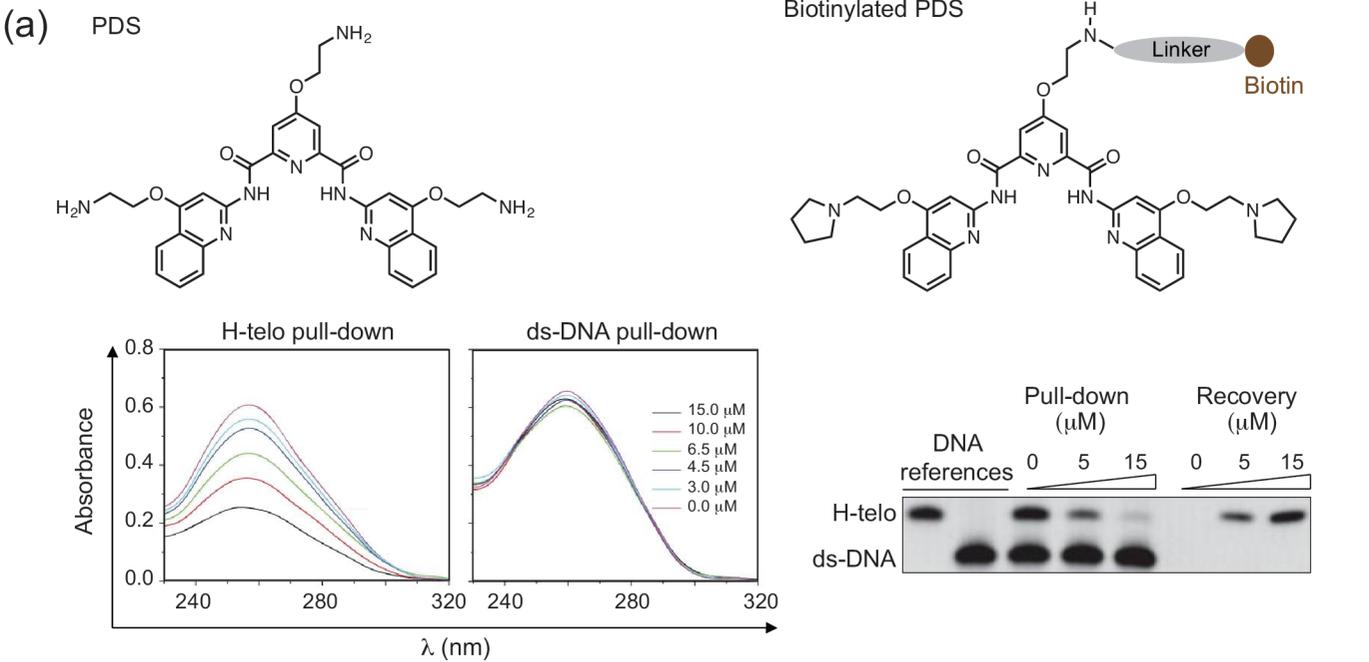
## Figure legends

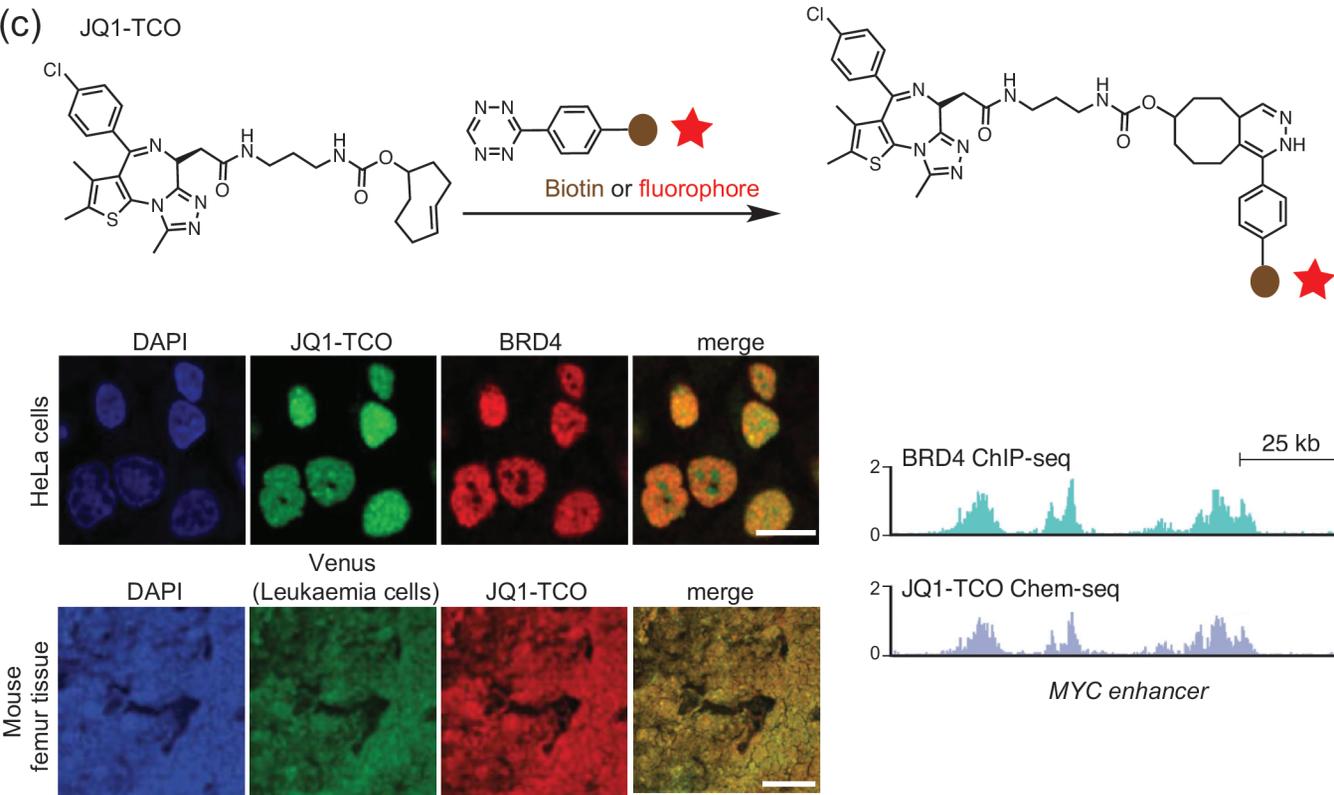
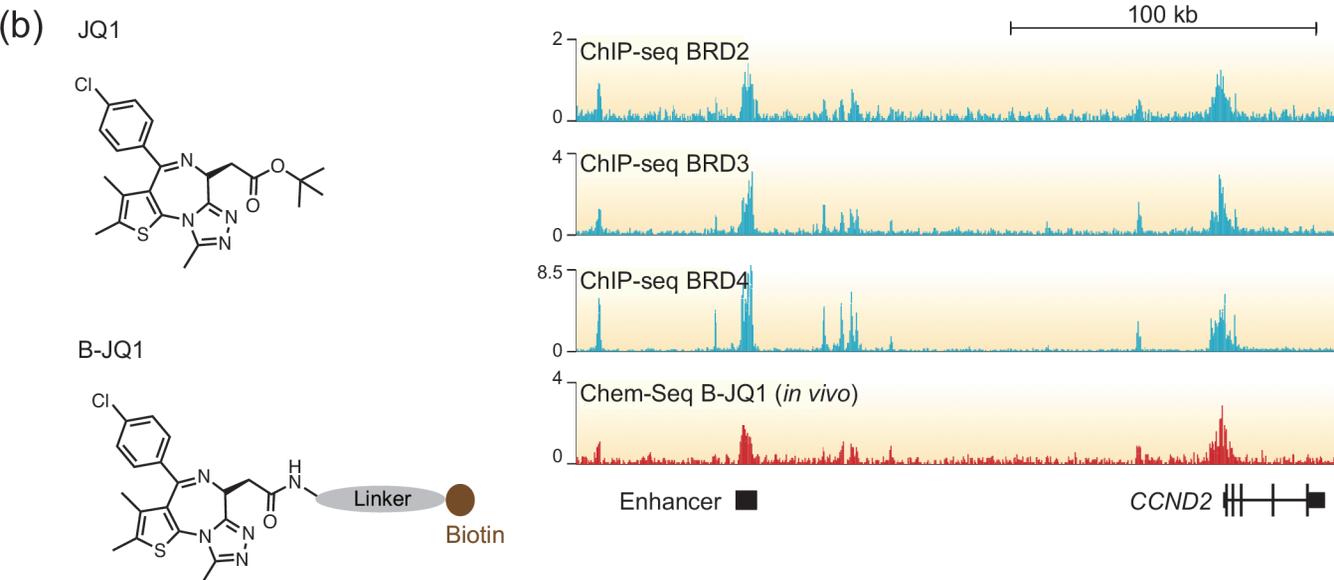
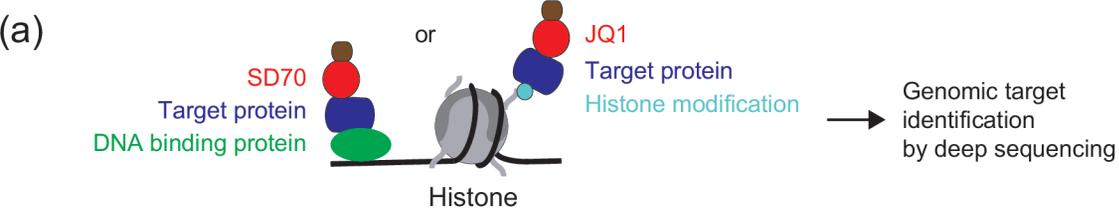
**Figure 1 | Whole-genome maps of G-quadruplexes.** (a) Molecular structures of pyridostatin (PDS) and biotinylated pyridostatin (top panel). UV absorbance measurements of remaining DNA in supernatants after pull-down using H-telo G4 or ds-DNA (lower panel, left). Gel showing selective G4 pull-down compared to ds-DNA pull-down (lower panel, right). Adapted from [13]. (b) Molecular structure of PDS labelled with a fluorophore, allowing the visualization of the small molecule in cells (left panel). Overlapping of fluorescently tagged PDS- $\alpha$  with the G4 binding helicase Pif1 $\alpha$  in U2OS human osteosarcoma cells (middle panel). Scale bar, 5  $\mu$ m. ChIP-Seq identified  $\gamma$ H2AX regions in oncogenes and tumor suppressors containing PQS clusters in MRC5-SV40 fibroblasts treated with PDS (right panel). Transcripts are shown, the purple bars correspond to putative G4 sequences determined bioinformatically. Adapted from [15]. (c) Schematic representation of the G4-seq method. Two sequencing runs, under normal and G4 stabilizing conditions, provide a reference map and detect G4-dependent polymerase stalling (upper panel). Identification of the base mismatches for the c-kit control sequence depicted in a heatmap plot of sequencing in Na<sup>+</sup>, K<sup>+</sup> and PDS conditions (lower panel). Each row is an independent sequenced template, and each column corresponds to the sequenced bases. Mismatches are shown in red. Adapted from [18]. (d) Schematic representation of the G4-ChIPseq method (upper panel). Example of G4 ChIP peaks for a 100-kb region in different cell lines (lower panel). Adapted from [23].

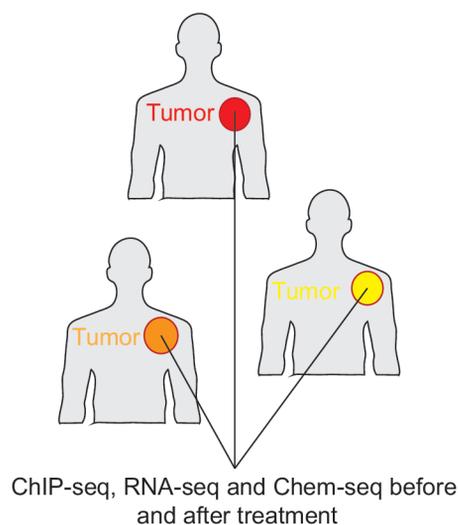
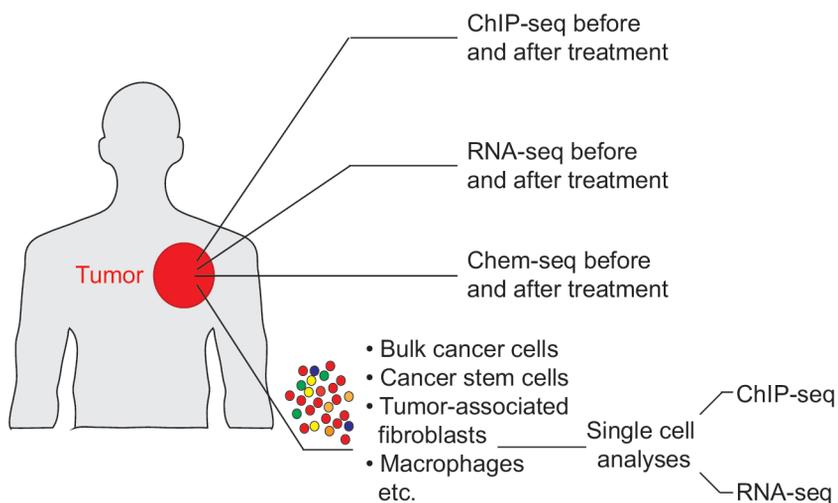
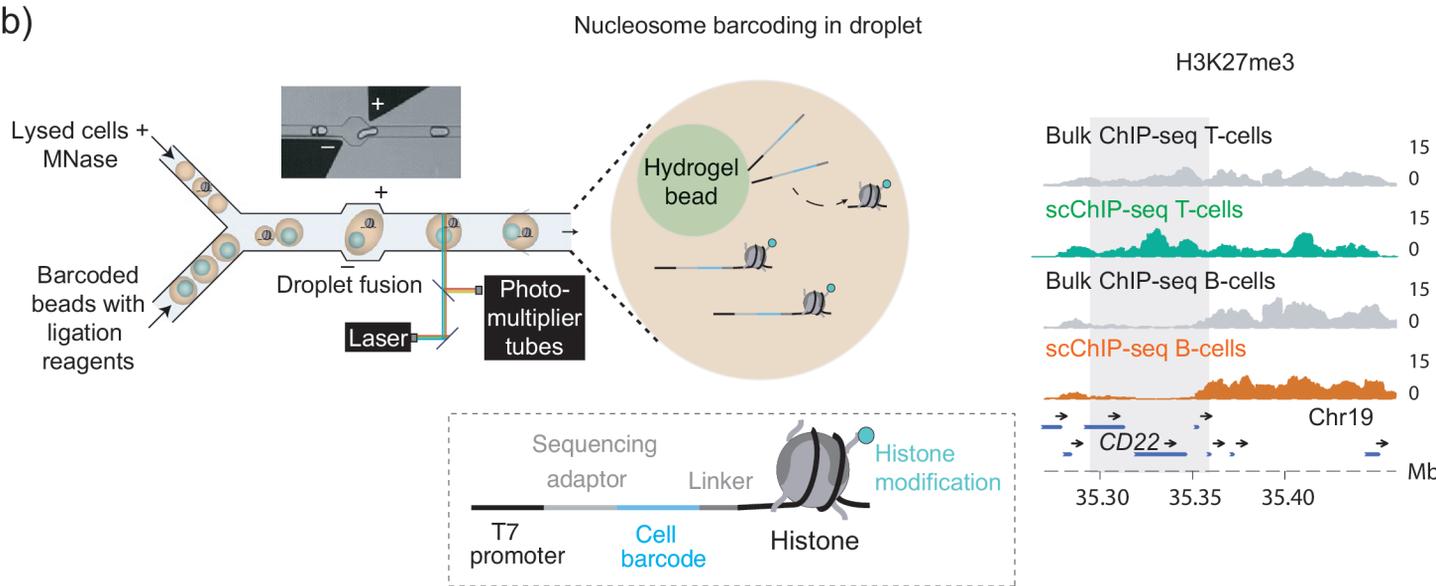
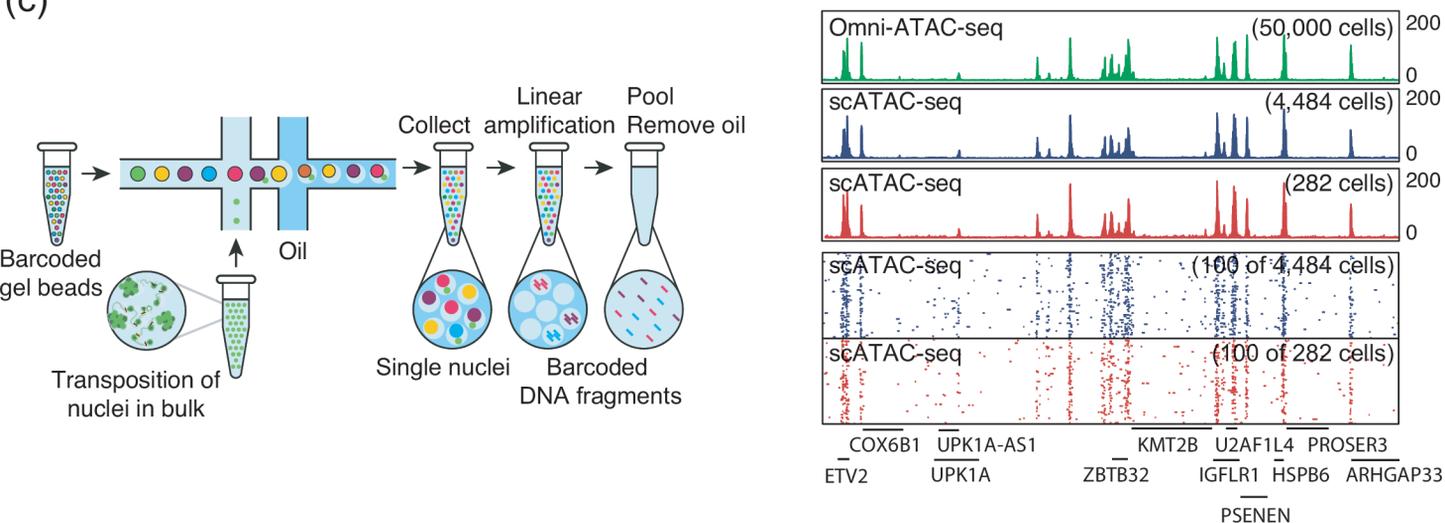
**Figure 2 | Whole-genome maps of JQ1 responses.** (a) Biotinylated derivatives of JQ1 or SD70 can be used to isolate and sequence genomic targets. (b) Molecular structures of JQ1 and

its biotinylated version B-JQ1 (left panel). Examples of high resolution genome enrichment data from ChIP-seq and Chem-seq methods for B-JQ1 and BRD2, BRD3 and BRD4 at the *CCND2* locus (right panel). Adapted from [2,3]. (c) Chemical labelling of JQ1-TCO with a biotin or a fluorophore (upper panel). Clickable compound JQ1-TCO can be visualized *in vitro* (HeLa cells, scale bar: 20  $\mu\text{m}$ ) and *in vivo* (mouse femur tissue, scale bar: 187  $\mu\text{m}$ ) (lower panel, left). Genome browser view of the *MYC* enhancer, comparing BRD4 ChIP-seq with click-seq using JQ1-TCO (lower panel, right). Adapted from [5].

**Figure 3 | Intra- and intertumoral heterogeneity and single-cell technology in cancer.** (a) Various sequencing approaches to decipher cell heterogeneity in cancer. (b) Epigenetic profiling using scChIP-seq on a single-cell basis. Overview of a microfluidics scChIP-seq workflow (left panel). Snapshot of differentially enriched *loci* of bulk profiles along with cumulative single-cell profiles for B-cells and T-cells. Differentially bound region is indicated in gray (right panel). Adapted from [47]. (c) Profiling of cancer cells on a single-cell level using scATAC-seq. Overview of scATAC-seq workflow (left panel). Snapshot of scATAC-seq results comparing different cell numbers (right panel). Adapted from [48].





**(a) Inter-tumor heterogeneity****Intra-tumor heterogeneity****(b)****(c)**

## Inter-tumoral heterogeneity

## Intra-tumoral heterogeneity

