Making drug discovery more efficient: predicting drug side effects in early screening efforts

Drug research and development is a complex and expensive process that begins with initial screening steps of candidate chemical compounds. Compounds that appear to have the desired potency against a specific cellular target or pathway are further evaluated. Candidate compounds that fail late in development or during clinical trials because of deleterious off-target effects are costly, and can be dangerous. Therefore drug developers not only need to ensure that a candidate compound is effective as a therapy, but also they need to predict any potential undesirable side effects due to off-target activities as early as possible in the drug discovery and development process.

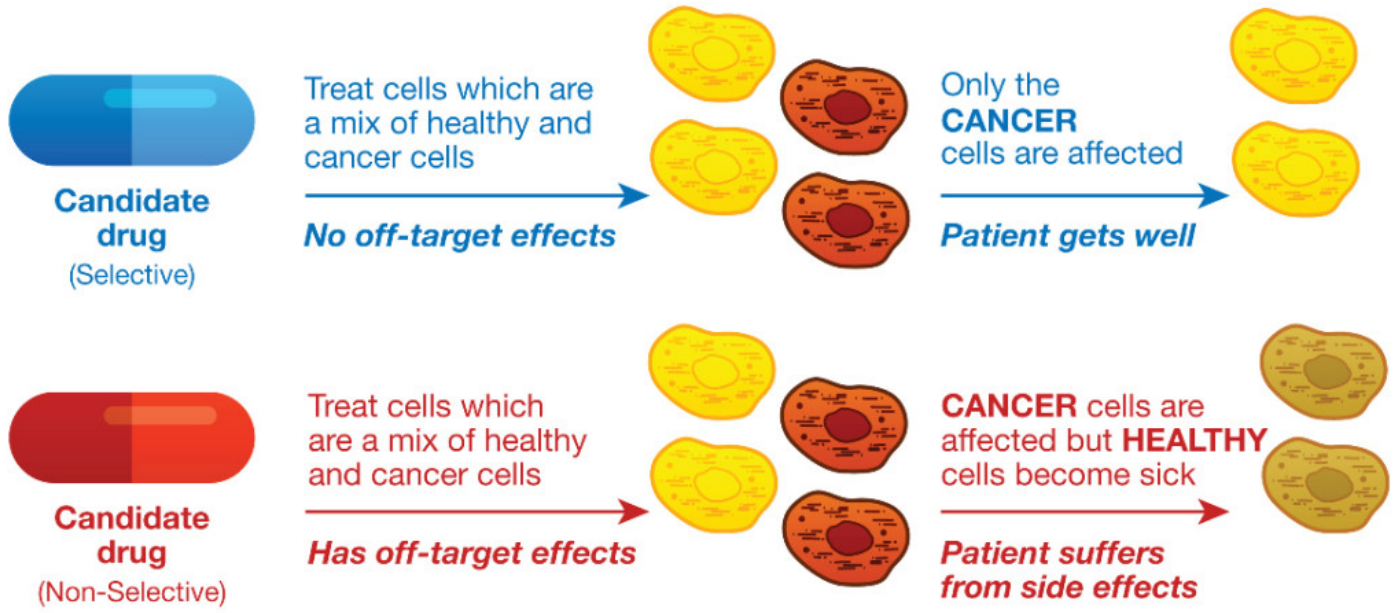

Fig.1. Effect of selective and non-selective drugs on cell health. Compounds that target kinases can be potent in destroying cancer cells. However, if such compounds are promiscuous towards non-target kinases, they will not only affect the cancer cells but also healthy cells, making them sick.

Cells carry out specific activities such as cell division, growth, and energy usage in response to signals from the environment, internal cues like DNA damage and even from other cells. These signals are perpetuated and amplified within the cell through biochemical events that modify proteins in the cell. One of the most common events of cell signaling is the addition of phosphate groups to proteins (phosphorylation), and phosphorylation is catalyzed by a specific group of

proteins called kinases.

Because kinases are one of the largest enzyme classes in the cell and they are involved in many critical cellular processes such as growth, energy use, and cell division, any disruption in their structure (mutations) or activity leads to diseases such as cancer, inflammation or diabetes. Therefore, kinases are often targeted in drug development efforts, and many small-molecule inhibitors of kinases have already been developed for treatment of different cancers. However, many candidate compounds that show great potential also often have off-target effects that make them unsafe as therapeutics (Fig. 1). In part this is because molecules that inhibit kinase activity tend to affect the highly conserved regions of these abundant proteins.

		SB203580	Gefitinib	Dasatinib	Tofacitinib	Roscovitine
TK-1	EGFR	99	2	64	102	94
	HER2	87	18	106	89	97
	HER4	96	28	13	103	101
	IGF1R	102	103	100	102	96
	InsR	98	97	94	97	101
	KDR	84	86	83	83	91
	PDGFRα	94	62	2	90	93
	PDGFRβ	85	85	1	77	92
TK-2	ABL1	98	97	0	106	107
	BRK	54	73	4	115	105
	BTK	99	83	5	101	111
	CSK	95	97	3	99	102
	FYN A	90	74	0	67	97
	LCK	90	74	2	89	92
	LYN B	95	75	0	97	96
	SRC	89	90	0	98	91
TK-3	AXL1	94	108	92	105	102
	EPHA1	79	84	1	102	97
	Fak	106	95	84	118	109
	ITK	94	102	101	99	100
	JAK3	87	99	113	0	94
	PYK2	106	133	134	122	134
	SYK	87	104	72	84	94
	TRKA	89	95	82	60	87
CMGC-2	CDK1/Cyclin A2	111	95	85	112	64
	CDK2/Cyclin A2	99	111	99	77	24
	CDK3/Cyclin E1	89	99	116	99	44
	CDK5/p25	97	89	104	100	26
	CDK5/p35	101	107	99	96	25
	CDK6/Cyclin D3	104	106	111	93	84
	CDK9/Cyclin K	110	100	93	104	41
	CLK3	90	106	92	98	98

>60% kinase activity | 20-60% kinase activity | <20% kinase activity

Fig. 2. Selectivity profile of kinase inhibitors generated by the bioluminescent kinase strip method. Compounds are profiled against 8 kinases at a time and percent kinase activity remaining is reported. An example of a specific kinase inhibitor such as tofacitinib is shown. Tofacitinib is a potent and selective JAK3 kinase inhibitor FDA approved for the treatment of rheumatoid arthritis.

Developing methods for profiling the effect of a target compound on the kinase activity (selectivity assessment) is challenging, and many of the current methods are cumbersome and still rely on radioisotopes. First, to profile the selectivity of a compound, many kinases must be assayed at the same time, and one kinase assay method that is suitable for one kinase may not be appropriate for other kinases. Second, developing and optimizing assays for each kinase in the selectivity profiling panel and maintaining the stability and supply of the enzymes for the assay is difficult.

Here we describe a pre-configured and standardized kinase profiling system that can be implemented as a cost-effective in-house screening assay which can be performed by any scientist in early evaluation of candidate compounds (Fig. 2). This system uses a universal kinase assay chemistry that can reliably probe kinase activity regardless of the nature of the substrate because it monitors the production of the universal kinase reaction product, ADP.

Such an assay allows drug developers to screen candidate compounds against a wide range of kinases early in the drug development process, potentially identifying problematic off-target effects. In this paper we describe previously unreported off-target effects for two reported kinase inhibitors. Additionally the same assay can be used to gain more information about the potency of a compound toward a particular targeted kinase or family of kinases later in the screening.

Drug discovery is complex and expensive, and being able to learn as much as possible about candidate compounds early in the screening process is critical to streamlining the process and ensuring that only the most viable compounds progress to later stages of drug development. The flexible bioluminescent kinase strips that we describe here allow researchers to fully understand the activity of their candidate drugs at an earlier stage than ever before.

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Publication

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