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How to cite

NOWAK-SLIWINSKA, Patrycja, SCAPOZZA, Leonardo, RUIZ ALTABA, Ariel. Drug repurposing in oncology: Compounds, pathways, phenotypes and computational approaches for colorectal cancer. In: Biochimica et Biophysica Acta. Reviews on Cancer, 2019, vol. 1871, n° 2, p. 434–454. doi: 10.1016/j.bbcan.2019.04.005

This publication URL: https://archive-ouverte.unige.ch//unige:120328
Publication DOI: 10.1016/j.bbcan.2019.04.005

BBA - Reviews on Cancer 1871 (2019) 434–454

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

Drug repurposing in oncology: Compounds, pathways, phenotypes and computational approaches for colorectal cancer

Patrycja Nowak-Sliwinska[a,b,*], Leonardo Scapozza[a], Ariel Ruiz i Altaba[c]

[a] School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, Geneva, Switzerland
[b] Translational Research Center in Oncohaematology, University of Geneva, Rue Michel Servet 1, 1211 Geneva 4, Switzerland
[c] Department of Genetic Medicine and Development, Faculty of Medicine, University of Geneva, Rue Michel Servet 1, 1211 Geneva 4, Switzerland

ARTICLE INFO

Keywords:
Repurposing in oncology
Colorectal cancer
Drug repositioning
Mechanism of action
Signaling pathways
On/off-target effects
Polypharmacology
Side effects
Omics
Phenotypes
Computational approaches

ABSTRACT

The strategy of using existing drugs originally developed for one disease to treat other indications has found success across medical fields. Such drug repurposing promises faster access of drugs to patients while reducing costs in the long and difficult process of drug development. However, the number of existing drugs and diseases, together with the heterogeneity of patients and diseases, notably including cancers, can make repurposing time consuming and inefficient. The key question we address is how to efficiently repurpose an existing drug to treat a given indication. As drug efficacy remains the main bottleneck for overall success, we discuss the need for machine-learning computational methods in combination with specific phenotypic studies along with mechanistic studies, chemical genetics and omics assays to successfully predict disease-drug pairs. Such a pipeline could be particularly important to cancer patients who face heterogeneous, recurrent and metastatic disease and need fast and personalized treatments. Here we focus on drug repurposing for colorectal cancer and describe selected therapeutics already repositioned for its prevention and/or treatment as well as potential candidates. We consider this review as a selective compilation of approaches and methodologies, and argue how, taken together, they could bring drug repurposing to the next level.

1. Introduction

The idea of using known and approved drugs for new indications has recently gained considerable momentum in many fields of medicine, especially in complex disorders [1,2]. This approach presents an important advantage, as compared to the "*de novo*" drug discovery process, by considerably reducing the cost and time to bring a new treatment to patients [3]. Drug repurposing, a term also referred to as repositioning or redirection, might be compared to giving a new use to an existing tool that already comes with a technical handbook: repurposed drugs have well-documented toxicity, pharmacology and drug-drug interaction parameters. Moreover, rescuing drugs that failed in one indication can deliver a potential drug candidate to another [4]. The current status of drug repurposing is collected in the reproDB site (http://apps.chiragjpgroup.org/repoDB/) [4]. It is encouraging that the EMA in Europe, as well as the NIH and the FDA in the USA have already launched drug repurposing programs to identify new uses for existing pipeline medications developed by the pharmaceutical industry [5,6].

1.1. Drug repurposing across medical fields

There are various classes of repurposed medications that include: (i)

Abbreviations: A-II, angiotensin-II; Ab, antibody; ACF, aberrant crypt foci; ARD, adverse drug reactions; AMPK, adenosine monophosphate-activated protein kinase; AT1R, angiotensin II type 1 receptor; ATC, Anatomical Therapeutic Chemical classification; CaPP3, Cancer Prevention Project 3; CHAT, cancer hallmarks analytics tool; CMap, Connectivity Map; COX-2, cyclooxygenase-2; CRC, colorectal carcinoma; DCF, Diclofenac; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; FAP, familial adenomatous polyposis; FMCM, Functional Module Connectivity Map; FFN, function-function networks; GSToP, gene-selection-by-trend-of-progression procedure; GWAS, Genome-Wide Association Studies; HERV, human endogenous retrovirus; KEGG, Kyoto Encyclopedia of Genes and Genomes; LBD, literature-based discovery; LINCS, Library of Integrated Network-Based Cellular Signatures; MANTRA, Mode of Action by NeTwoRk Analysis; MRC, Medical Research Council; NSAID, non-steroidal anti-inflammatory drug; NTID, narrow therapeutic index drug; OS, overall survival; PFS, progression free survival; Pl3K, phosphatidylinositol 3-kinase; POG, Personalized OncoGenomic; PREDICT, PREdicting Drug IndiCaTions; RAR α, retinoic acid receptor alpha; ReDo, Repurposing Drugs in Oncology; RRM2, human ribonucleotide reductase 2; SEA, Similarity Ensemble Approach; sL^A, sialyl Lewis-A antigen; SMILE, simplified molecular-input line-entry system; SVM, Support Vector Machine; TKI, tyrosine kinase inhibitors; TOP2A, Topisomarase 2-α; USPSTF, U.S. Preventive Services Task Force

* Corresponding author at: School of Pharmaceutical Sciences, University of Geneva and University of Lausanne, Geneva, Switzerland.
E-mail address: Patrycja.Nowak-Sliwinska@unige.ch (P. Nowak-Sliwinska).

https://doi.org/10.1016/j.bbcan.2019.04.005
Received 18 February 2019; Received in revised form 9 April 2019; Accepted 15 April 2019
Available online 26 April 2019

Table 1
Selected examples for successfully repurposed medications in different fields.

Drug	Group	Original indication	Repurposed indication	Refs.
Acetylsalicylic acid (Aspirin™)	2-Acetoxybenzoic acid	Analgesic; antipyretic to reduce fever	Inflammation; prevention in transient ischemic attack or heart attack, strokes	[7–9]
Amantadine (Symmetrel ™)	Anticholinergic-like agent	Influenza	Parkinson's disease	[10]
Dexmecamylamine (TC-5214)	Nicotinic receptor modulator	Central nervous system disorders	Depression	[11]
Exenatide (Byetta™)	Glucagon-type peptide-1 agonist	Diabetes type II	Non-diabetic obesity	[12]
Galantamine (Nivalin™)	Acetylcholinesterase inhibitor	Polio, paralysis	Alzheimer's disease	[13]
Mecamylamine (Inversine ™)	Nicotinic receptor antagonist	Hypertension	ADHD, depression	[14]
Methotrexate (Trexall ™)	Antifolate	Cancer	Rheumatoid arthritis or psoriasis	[15]
Mifepristone (Mifegyne ™)	Glucocorticoid receptor type II antagonist	Pregnancy termination	Psychotic major depression, Cushing's syndrome	[16]
Sildenafil (Viagra™)	Phosphodiesterase inhibitor	Anginal	Penile erections	[17]
Thalidomide (Thalomid ™)	(N-Phthalimido) glutarimide	Sedative; morning sickness in pregnancy	Refractory multiple myeloma	[18]
Zidovudine (Retrovir ™)	Dideoxynucleoside	Cancer	HIV/AIDS	[19]

Drugs that were shelved due to toxicity when used for a given indication but are not toxic at lower doses and efficient alone or in combination for a different indication. For instance, thalidomide, which was used for morning sickness during pregnancy but eventually withdrawn from the market because of its teratogenicity (producing limb malformations), is now repurposed for refractory multiple myeloma excluding pregnant women. (ii) Drugs whose off-target effects are prioritized as the main indication, of which the development of sildenafil (Viagra™) is a popular example (Table 1). This compound was developed as an anti-anginal drug but eventually found a useful repositioning for erectile dysfunction, making it a sales megahit [17]. Similarly, exenatide (Byetta™) (Table 1), originally used for type II diabetes, was repurposed to treat non-diabetic obese patients due to off-target weight loss [12], thus helping in the fight against the current overweight epidemic.

1.2. Drug repurposing: logic, pipeline, process and pitfalls

Drug repurposing is based on the principle of polypharmacology, i.e. a paradigm in drug discovery where one drug with multiple targets and off-target effects may present multiple modes of action [20]. In fact, this may be the norm for all compounds as coined by Paracelsus (1493–1541) in his famous phrase: *"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison"*. Polypharmacology might thus be exploited in the search for more effective and less toxic treatment designs. Even more importantly, the interactions of drugs with 'off-target' proteins used to be considered as undesirable molecular promiscuity and responsible for undesirable side effects. However, this promiscuity may very well be intrinsic to a medication's therapeutic efficacy. 'Hybrid vigour' may thus have found another meaning at the pharmacological level.

The great complexity and redundancy (and thus consistency) of biological systems, together with the varying etiologies and pathologies of disease and our still limited knowledge of target function and behaviour, leaves any answer to the question of how drugs actually achieve clinical performance in patients incomplete. Tyrosine kinase inhibitors (TKI) are a good example of such drugs. Although they have been greatly improved to have potent selectivity, many have additional effects on other kinases and beyond their target family [21,22], thus displaying an intrinsic polypharmacology often favorable for their clinical efficacy [23].

For it to be generally successful, a polypharmacological repurposing approach needs the systematic integration of the scientific data derived from different drug discovery and mechanistic disciplines. These include *in silico* modeling, synthetic chemistry, *in vitro* screens, systems *pharmacology* approaches, *in vitro* and *in vivo* functional phenotypic analyses with human tumor cells (using for instance mouse xenografts [24] or organoids [25]), and most importantly clinical studies in patients with different genetic backgrounds [17].

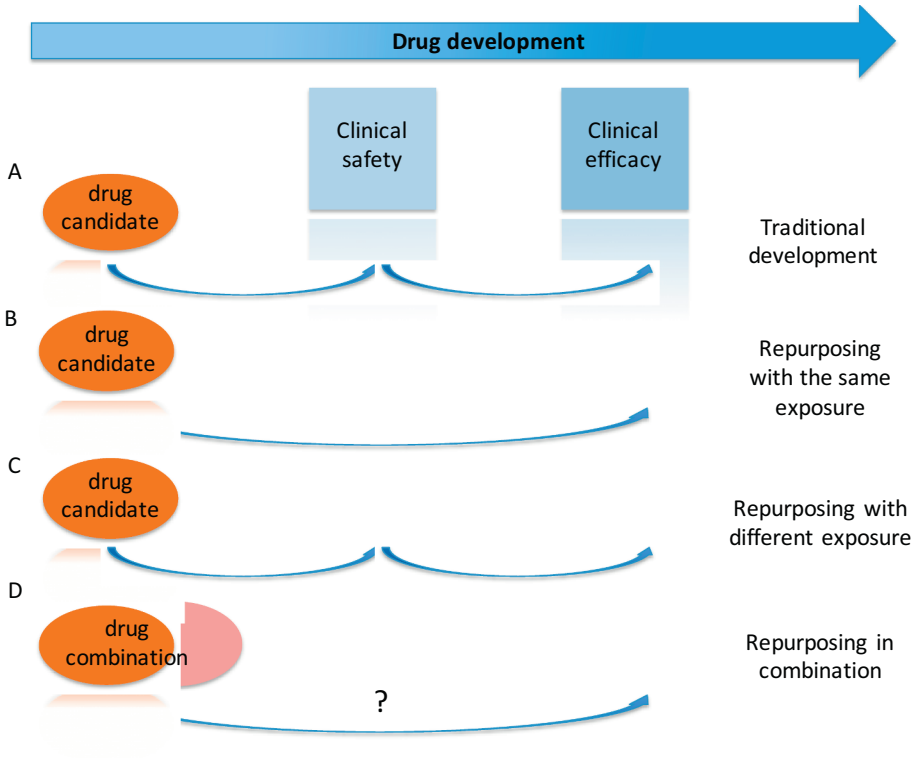
An essential point to take into consideration is the cost of drug development. Drug repurposing can save time, effort and money as compared to the classical *de novo* development of drugs [26], in which the time between discovery and clinical trials is of 9 years on average, the success rate of less than 10 % and the average cost per drug to the patient of several hundred million dollars [1]. In contrast, drug repurposing can take 3–4 years to clinical trials [1] and cost only a fraction of the amount needed to test a new drug class in patients [27,28]. This important cost-saving opportunity was enthusiastically welcomed by various national funding organizations and pharmaceutical industries. In 2014 almost 70 drugs de-prioritized at various development stages, mostly to the lack of activity, were made available for repurposing via the coalition of the British Medical Research Council (MRC) and seven pharmaceutical companies [28,29].

Nevertheless, while the repositioning of drug candidates with good safety and toxicology profiles can be quickly approved for another indication using the same administration route, the issue of ultimate efficacy remains the same [30]. The overall success rate is less than 6% [27], which is not significantly different from that of *de novo* developed oncological drugs, which is at 5% [30]. Indeed, it is important to note that lack of drug efficacy remains the main reason for attrition (30%) during clinical trials [30] (Scheme 1). This lack of efficacy seems higher in therapeutic areas in which animal models recapitulate less pertinently the human phenotype and thus, are less predictive of the patient situation [30]. Furthermore, if different exposures routes, e.g. systemic vs. local, are needed as compared to the original indication for the repurposed drug, dose-escalation, pharmacokinetic and toxicology studies will most likely be required.

Therefore, alongside developing general schemes for drug repurposing, major efforts need to be made to improve efficacy, and this largely relates to the choice of phenotypes chosen to monitor drug activity in pre-clinical model systems. Highly relevant and reliable phenotypes thus need to be tracked with custom-made assays using genetic benchmarks and epistatic analyses. This should involve testing efficacy and specificity in model systems through chemical genetics, taking into account the level of specificity of different drugs. In addition to efficacy, the determination of the therapeutic window is essential to ascertain the benefits of the drugs and to provide treatment strategies.

1.3. A snapshot of colorectal cancer and treatments: problems and opportunities

As a relevant example, we highlight here the case of colorectal cancer (CRC). This cancer is common in Western and 'westernized' societies and display increasing incidence [31]. Whereas early detection and complete surgical resection at the polyp/adenoma stages can hugely influence overall survival [32], the situation is different for local or



Scheme 1. Drug development scheme. A. Traditional drug development. B. Drug candidates with good clinical safety profiles can be repurposed relatively easily when the same, delivery route is used. C. Drug candidates repurposed using different routes than in the original indication require safety clinical testing. D. Repurposed drug combinations, depending on on-target/off-target characterization, might or might not (therefore the question mark) require a clinical safety step to reach clinical efficacy test.

invasive CRC. About 25–30% of confirmed CRC cancers without detectable local or distal invasion will eventually develop metastases and there is currently no cure for metastatic disease [33]. For this reason, adjuvant treatments are often given around the time of surgery to attempt to eliminate remaining cancer cells. Such residual cell populations appear to be enriched for cancer stem cells [34]. Blocking signaling pathways maintaining stemness is thus a rational goal to avoid recurrence as well as to block tumor growth and metastasis although so far there are no viable direct, small-molecule anti-stemness therapies.

A second issue relates to the finding that patients with metastases often present multiple dissimilar tumors. The heterogeneity of metastases and their underlying molecular mechanisms, including tumor evolution and selection, thus poses a large problem, and this may underlie the observed resistance to first line chemotherapy in metastatic CRC (mCRC) patients. Current chemotherapy based on the principle of "one fits all", even with drug combinations (see below), does not work efficiently and the response rates are low, in the range of approx. 20% [35], likely due, in part, to tumor heterogeneity.

Molecularly, the precise knowledge of driving mutations in the tumor bulk representing early events (e.g. > 80% loss of APC, 40% gain of oncogenic function of KRAS and 5–15% gain of oncogenic function of BRAF [36,37]) has not yet been translated in curative therapies. General cytotoxic chemotherapeutic regiments, as barbaric as these may seem in the future, are fruit of decades of work and are currently the standard first- and second-line treatments for patients with mCRC disease (in adjuvant or even metastatic settings), but have also not yielded great results. Usual combinations are fluoropyrimidine (5-FU or oral capecitabine) with either oxaliplatin (FOLFOX, i.e. folinic acid, 5-FU, and oxaliplatin or XELOX, i.e. capecitabine and oxaliplatin) or irinotecan (FOLFIRI, i.e. folinic acid, 5-FU, and irinotecan or XELIRI, i.e. capecitabine and irinotecan) [38–41]. Such treatments, currently afford only an overall survival prolongation of several months, often with heavily compromised quality of life [32].

The approval of agents that target angiogenic signaling, i.e. anti-vascular endothelial growth factor antibody (Ab) (bevacizumab, Avastin™) [42], a chimeric anti-epidermal growth factor receptor (EGFR) Ab (cetuximab, Erbitux™) [43], a fusion protein containing VEGF binding domains of VEGFR1 and -2 (aflibercept, Zaltrap™), and a multikinase small molecule inhibitor (regorafenib, Stivarga™) provided new hope [44,45]. Unfortunately, these treatments do not stop relapse [46].

In mCRC patients, the response rate to first line treatment is no more than 50% and this is independent from the chemotherapeutic drug combination used. Moreover, the majority of these patients will develop drug-resistance and will succumb to the disease [38]. Thus, the goal now is to develop and repurpose drugs to combat multiple tumors in an individual patient that display genetic and phenotypic heterogeneity.

As mentioned above, a major issue in the treatment of cancer is resistance to a given drug, observed with anti-mitotic or toxic chemotherapeutics as well as with targeted compounds [47,48]. One example of the latter is the resistance to SMOOTHENED (SMO) inhibitors in medulloblastoma resulting in unresponsive, progressive disease [49]. The finding that resistant clones can already exist within heterogeneous primary tumors (SMO example above) further highlights the central problems of tumor cell heterogeneity in cancer.

Finally, cancer prophylaxis in the healthy but at-risk population is becoming a central theme in public health and we foresee that drug repurposing will be an important approach in this context. The majority of colorectal carcinomas develop from adenomas through step-wise genetic changes [50]. When adenomas grow or become dysplastic the risk of subsequent bona fide cancer is very high [51]. Therefore, targeting early, undetected adenomas and polyps present in the at-risk general population (e.g. people after the age of 50 years or with genetic predispositions) might be one of the preferred strategies to prevent cancer. Overall, while promising, few options for prevention from the available clinical data exist. e.g. the use of aspirin in individuals at adequately high cardiac risk (see chapter below). A number of molecules, however, could enter or have entered clinical trials to clarify if they could be used as preventive agents adhering to the additional goals of being safe with good patience compliance and cost-effective.

In the first part of this review, we seek to outline the progress, challenges and opportunities of drug repurposing for CRC treatment

Table 2
Selected drug-repurposing candidates for CRC prevention and/or treatment (in the order of increasing evidence).

Drug	Pharmacological class	Original indication	Evidence			Attrition	CRC			Refs.
			In vitro	*In vivo*	Clinical		Prevention	Treatment		
								Primary	Metastatic	
Prevention										
Clotam (Clotam Rapid™)	NSAID	Pain		+			+			[52]
Celecoxib (Celebrex™)	NSAID	Pain, inflammation			+		+			[53,54]
Rofecoxib (Vioxx™)	NSAID	Osteoarthritis, acute pain, dysmenorrhea			+	+	+			[55]
Metformin (Glucophage™)	Biguanide	Diabetes type II			+		+			[56–58]
Sulindac (Clinoril™)	NSAID	Pain, fever, inflammation			+		+			[59]
Acetylsalicylic acid (Aspirin™)	Salicylate	Pain, fever			+		+			[60–62]
Treatment										
Amantadine (Symmetrel™)	Dopamine agonist	Viral infections, Parkinson disease	+					+		[63]
Pleconaril (Picovir™)	Capsid inhibitor	Viral infections, Parkinson disease	+				+			[63]
Disulfiram (Antabuse™)	Aldehyde dehydrogenase inhibitor	Alcoholism	+						+	[64,65]
Ivermectin (Stromectol™)	Anthelmintic drug	Parasite infections	+	+			+			[66]
Diclofenac (Voltaren™)	NSAID	Pain		+				+	+	[67–70]
Captopril (Capoten™)	Angiotensin converting enzyme (ACE) inhibitor	Hypertension		+					+	[71,72]
Irbesartan (Avapro™)	Angiotensin receptor blocker	Hypertension		+					+	[73,74]
TCV116 (Candesartan™)	Angiotensin receptor blocker	Hypertension		+					+	[75]
Mesalamine (Mesalazine™)	Aminosalicylate	Inflammatory bowel diseases		+	+			+		[76]
Cimetidine (Tagamet™)	Anti-histamine; H2-histamine receptor antagonist	Dyspepsia, heartburn, peptic ulcers			+				+	[77–79]
Aspirin (Acetylsalicylic acid)	Salicylate	Pain, fever	+	+	+			+	+	[66,80,81]
Epirubicin (Ellence™)	Anthracycline	Node-positive breast cancer			+				+	[82,83]
Metformin (Glucophage™)	Biguanide	Diabetes type II		+	+			+	+	[84,85]
Treatment (based on computational/integrated approaches)										
Phenoxybenzamine (Dibenzyline™)	Alfa blocker	Hypertension	+							[86]
GW-8510	Cyclin kinase 2 inhibitor	RRM2 (human ribonucleotide reductase 2) inhibitor	+					+		[86,87]
Primaxin (Imipenem™)	Carbapenem (thienamycin) + dehydropeptidase I inhibitor	Bacterial infections	+					+		[86]
Enilconazole (Imazalil™)	Fingicide	Fungal infections		+					+	[88]
Citalopram (Celexa™)	Selective serotonin reuptake inhibitor	Depression		+					+	[88]
Troglitazone (Rezulin™)	Thiazolidinedione	Diabetes		+		+			+	[88]

and prevention, both as monotherapy and in combination. For instance, we discuss selected examples of drugs that could be repurposed based on chemical genetic evidence and suitable *in vitro/in vivo* phenotypic analyses. This includes the widely used anti-parasitic drug ivermectin repositioned as a WNT signaling-response blocker. In the second part of the review we emphasize the new emerging computational tools that could (i) integrate knowledge and pinpoint new drug candidates, as well as (ii) accelerate effective drug discovery through drug repositioning that (iii) indicate promising methods for combinatorial therapies.

2. Selected repurposing candidates for the treatment and/or prevention of CRC

Non-cancer medications may represent a relatively untapped source of novel cancer therapies. A number of them are discussed below in the context of prevention and/or treatment of CRC (see also Table 2). The agents are presented in the increasing order of evidence of their activity in CRC, i.e. supported with *in vitro* data, via results obtained in animal models, to finish with epidemiological and clinical data. Many of these have been identified by the Repurposing Drugs in Oncology project (ReDO) [23], an international collaboration between the Anticancer Fund in Belgium and American GlobalCures. For the convenience of the reader the agents' chemical structures as well as the simplified molecular-input line-entry system (SMILE) are listed in Table 3.

One must note that lacking the ability and will to test compounds in humans directly, all 'models' have shortcomings. For instance, the use of rodents instead of primates (humans), or of epithelial tumor cells without the interacting immune, neural and hormonal systems. Moreover, the results obtained only in laboratory cell lines may have additional shortcomings that may further impair their translation into *in vivo* conditions as these cells are cloned, have been passed over thousands of generations and have adapted to growth on plastic in high-serum media. Additional problems of cells in culture include the use of high drug concentrations that may not be able to be reached in patients, or the use of cells with experimentally-induced drug resistance. Ideally it would be necessary to collect phenotypic data at multiple overlapping levels, for instance from molecular, to cellular, to primary culture, to whole animal, to distinct patient populations, in order to increase the confidence that results will be positively translatable to individual patients.

2.1. CRC prevention

2.1.1. In vivo studies

2.1.1.1. Clotam. A tolfenamic acid (TA) from the NSAID class, Clotam, was used initially for migraine-related pain treatment. Clotam interacts with COX-independent targets such as SURVIVIN, β-CATENIN, CYCLIN D1 and MATRIX METALLOPROTEINASE 7 [52]. In recent preclinical studies in the polyposis of a rat colon (Pirc) model, Ertem *et al.* performed transcriptome profiling of colorectal adenomas and compared the results with those obtained from adenoma patients [52]. From the list of identified genes in transcriptomic profiling the authors indicated *Mmp7, S100a9, Nppb and Aldh1a3* as downregulated in colon tumors after TA treatment and suggested them being candidate biomarkers in CRC prevention. Clotam is therefore a promising drug candidate that needs further investigation to prove its utility for CRC treatment.

2.1.2. Clinical evidence

2.1.2.1. Celecoxib and rofecoxib. The use of COX-2 inhibitors celecoxib for CRC prevention was reported in several clinical trials. A group of 77 patients with familial adenomatous polyposis (FAP), treated with celecoxib (400 mg/twice daily for 6 months) led to a significant reduction in the number of colorectal polyps [53,54]. However, the long-term result of treatment with celecoxib was associated with frequent cardiovascular events and gastrointestinal side effects despite the efficacy in the adenoma recurrence trial [89,90]. Similar thrombotic and cardiovascular adverse results were observed after treatment with rofecoxib, another COX-2 inhibitor, leading to the removal of this drug from the market worldwide [55]. A recent clinical trial in FAP patients showed that celecoxib administered together with difluoromethylornithine (an irreversible inhibitor of ornithine decarboxylase) was not superior to celecoxib alone [91]. Therefore, it is not entirely clear if the COX-2 inhibitors as monotherapies would be further developed for CRC prevention.

2.1.2.2. Metformin. Multiple studies including epidemiological studies, preclinical research and clinical trials suggested that metformin also lowered the risk of developing CRC. Epidemiological studies reported various, often contradictory evidences on a risk of CRC ([58,92,93]). Those discrepancies might be due to the time-related biases such as immortal time bias [94]. The recent cohort study with over forty seven thousand participants designed to minimize those biases revealed that long-term use of metformin (≥5.0 years) appeared to be associated with reduced risk of colorectal cancer in the full population [95].

At the preclinical level, numerous *in vivo* in various animal models, both genetic and/or sporadic, the latter being induced by a carcinogen [96]. Administration of metformin significantly suppressed the number of intestinal polyps formed in both models [97,98], not only murine, but also in rats [99].

Based on the epidemiological and preclinical data, it is suggested that metformin may indeed have a chemoprotective efficacy upon CRC. Therefore, various trials set their endpoints on the correlation of the number of aberrant crypt foci, small lesions that develop in the earliest stage of colorectal carcinogenesis, that were reported to be precursor lesions for human colorectal carcinogenesis, and the efficacy of candidate chemoprotective agents. 26 non-diabetic patients with aberrant crypt foci (ACF) were randomized to two arms treated with metformin (250 mg/d) or no treatment. At 1 month, the patients in metformin treated arm a significant decrease in the mean number of ACF, whereas not change was observe in the control arm [100]. The shortcoming of this trial, however is a short observational time of only 1 month. Since CRC incidence is fairly low in the general population, and requires long-term observational period, the incidence of adenomas might be rather unsuitable endpoint for chemoprevention trials. A multicenter, double-blind, placebo-controlled, randomized controlled trial was conducted in non-diabetic adults patients in order to evaluate the chemopreventive effect and safety of metformin (250 mg/day) against metachronous colorectal polyps. This relatively low dose of metformin, as compared to other trials, was proposed in order to limit possible side effects correlated to high-dose metformin [101]. The incidence of total polyps (i.e. adenomas and hyperplastic polyps) in the metformin treated group was significantly lower than that in the placebo group and the side effects such as diarrhea or abdominal pain were very mild [102]. Since in this study there was no CRC detected in any subjects in the 1 year follow-up colonoscopy, further long-term studies minding gender and age differences, on bigger patient population or better-defined cohorts are needed to truly validate the efficacy of metformin for the prevention of CRC.

2.1.2.3. Sulindac. A COX inhibitor of the arylalkanoic acid class, used initially for treating pain, fever, and inflammation, sundilac has been extensively studied for chemoprevention in FAP patients [89]. FAP patients are at high risk for duodenal neoplasia, with duodenal adenomas eventually forming in over 50% of participants and duodenal adenocarcinoma occurring in approx. 12% [103,104].

A patient with FAP would commonly have to be followed with at least some adenomas left *in situ*, thus providing an opportunity to test a drug for adenomas regression. Therefore, the FAP can be considered a "training ground" for drugs that would potentially slowed down or reverse the adenoma-carcinoma sequence [89].

Table 3
Chemical structures and simplified molecular-input line-entry systems (SMILE).

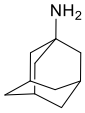
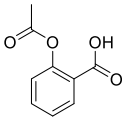
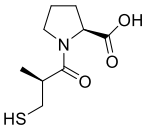
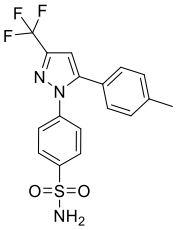
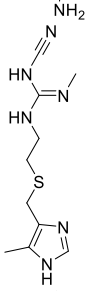
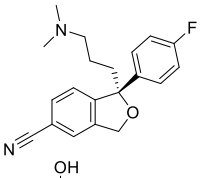
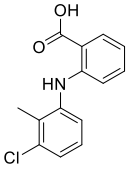
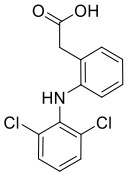
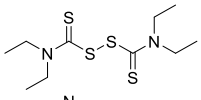
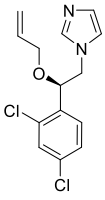
Name	Chemical structure	Smile code
Amantadine (Symmetrel™)		NC1(C[C@@H]2C3)C[C@@H]3C[C@@H](C2)C1
Acetylsalicylic acid (Aspirin™)		O=C(C(C=CC=C1)=C1OC(C)=O)O
Captopril (Capoten™)		SC[C@@H](C)C(N(CCC1)[C@@H]1C(O)=O)=O
Celecoxib (Celebrex™)		O=S(N)(C(C=C1)=CC=C1N(C(C(C=C2)=CC=C2C)=C3)N=C3C(F)(F)F)=O
Cimetidine (Tagamet™)		CC1=C(N=CN1)CSCCN/C(NC#N)=N/C
Citalopram (Celexa™)		FC(C=C1)=CC=C1[C@@]2(CCCN(C)C)OCC3=C2C=CC(C#N)=C3
Clotam (Clotam Rapid™)		ClC1=C(C)C(NC(C=CC=C2)=C2C(O)=O)=CC=C1
Diclofenac (Voltaren™)		ClC(C=CC=C1Cl)=C1NC2=C(CC(O)=O)C=CC=C2
Disulfiram (Antabuse™)		S=C(N(CC)CC)SSC(N(CC)CC)=S
Enilconazole (Imazalil™)		CClC1=C([C@H](CN2C=NC=C2)OCC=C)C=CC(Cl)=C1

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Table 3 (*continued*)

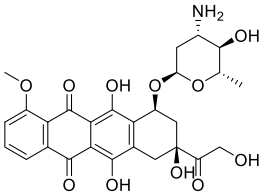
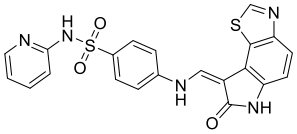
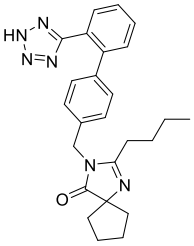
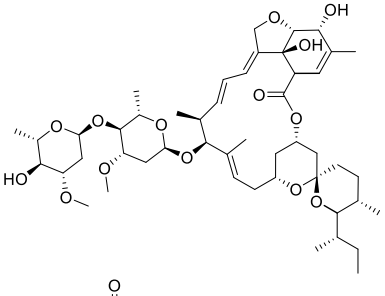
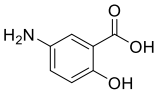
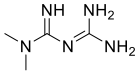
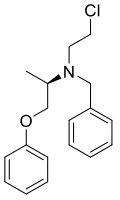
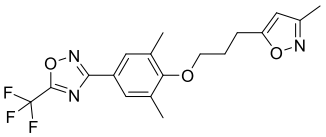
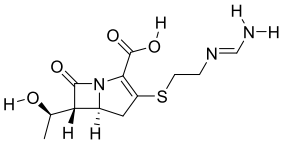
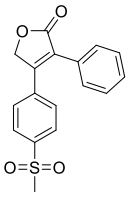
Name	Chemical structure	Smile code
Epirubicin (Ellence™)		O=C1C2=C(O)C([C@@H](O[C@@H]3O[C@@H](C)[C@H](O)[C@@H](N)C3)C[C@@](C(CO)=O)(O)C4)=C4C(O)=C2C(C5=C1C(OC)=CC=C5)=O
GW-8510		O=S(C1=CC=C(N/C=C2C(NC3=C\2C(SC=N4)=C4C=C3)=O)C=C1)(NC5=NC=CC=C5)=O
Irbesartan (Avapro™)		O=C1N(CC(C=C2)=CC=C2C(C=CC=C3)=C3C4=NNN=N4)C(CCCC)=NC15CCCC5
Ivermectin (Stromectol™)		O=C([C@@H]1C=C(C)[C@@H](O)[C@@H]2[C@@]1(O)/C(CO2)=C/C=C/[C@H](C)[C@H](O[C@@H]3O[C@@H](C)[C@H](O[C@@H]4O[C@@H](C)[C@H](O)[C@@H](OC)C4)[C@@H](OC)C3)/C(C)=C/C5)O[C@@H]6C[C@@]7(O[C@H]([C@@H](C)CC)[C@@H](C)CC7)O[C@H]5C6
Mesalamine (Mesalazine™)		O=C(O)C1=CC(N)=CC=C1O
Metformin (Glucophage™)		N=C(N(C)C)/N=C(N)/N
Phenoxybenzamine (Dibenzyline™)		ClCCN(CC1=CC=CC=C1)[C@@H](C)COC2=CC=CC=C2
Pleconaril (Picovir™)		FC(F)(C1=NC(C2=CC(C)=C(C(C)=C2)OCCCC3=CC(C)=NO3)=NO1)F
Primaxin (Imipenem™)		O=C1N2[C@@](CC(SCC/N=C/N([H])[H])=C2C(O[H])=O)([H])[C@@]1([H])[C@H](C)O[H]
Rofecoxib (Vioxx™)		O=C1OCC(C(C=C2)=CC=C2S(=O)(C)=O)=C1C3=CC=CC=C3

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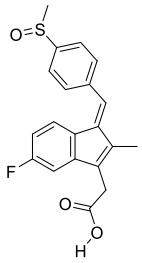
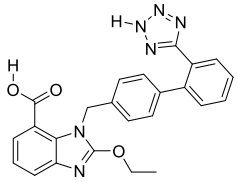
Table 3 (*continued*)

Name	Chemical structure	Smile code
Sulindac (Clinoril™)		FC1=CC(C(CC(O[H])=O)=C/2C)=C(C=C1)C2=C/C(C=C3)=CC=C3S(C)=O
TCV116 (Candesartan™)		O=C(C1=C(C(N=C2OCC)=CC=C1)N2CC3=CC=C(C4=CC=CC=C4C5=NN([H])N=N5)C=C3)O[H]

The FAP commonly undergo surgery as first line intervention at a relatively early age, often combined with prevention treatments with NSAIDs. In the randomized, double-blind, placebo-controlled study of 22 patients with FAP, sulindac alone was given (150 mg orally twice a day) for 9 months with the primary endpoint of evaluation of number and size of the polyps. A statistically significant decrease in the mean number of polyps and their mean diameter occurred, as compared to placebo arm. However, after the treatment with sulindac was stopped, both the number and the size of the polyps increased in sulindac-treated patients. Both parameters remained, however, significantly lower than the values at base line suggesting incomplete effect of sulindac and no complete regression was observed. Those data suggest the necessity of sulindac combination with other agents [59]. Other clinical trials with sulindac alone were conducted with other sulindac dosing, polyp burden description, or polyp stage [105], therefore impairing the possibility of direct comparison.

The prospective randomized study of sulindac versus calcium and calciferol administrated for 6 months for upper gastrointestinal polyps in FAP patients, revealed no regression in duodenal adenomas, which at least in part, was attributed to the small sample size [106].

A combination of sulindac with the TKI erlotinib, as shown in a double-blind, randomized, placebo-controlled trial enrolling 92 participants with FAP, resulted in a significantly lower duodenal polyp burden at 6 months with grade 1 (mild) or 2 (moderate) toxicities as compared to placebo arm [107]. Another randomized, double-blind placebo-controlled trial with enrolled three hundred seventy five patients with duodenal adenoma regression in patients with FAP. The primary outcome was change in total polyp burden at 6 months for a combination of a low dose of oral difluoromethylornithine plus a low dose of sulindac [108]. Recurrent adenomatous polyps were markedly reduced by the drug combination with few adverse effects.

In 2013 a phase III double blind placebo-controlled trial of Sulindac and Eflornithine to prevent recurrence of high-risk adenomas and second primary stage 0 to III of CRC patients was initiated (NCT01349881). Enrolled patients underwent curative surgery and were treated with Sulindac or Eflornithine for 36 months with a subsequent 5-year follow up period. The results are pending.

2.1.2.4. Acetylsalicylic acid (Aspirin™). The link between inflammation and cancer is long known [109] and is been further supported by the observation that anti-inflammatory and immune therapies can be applied against cancer [110,111]. In this context, aspirin as well as other non-steroidal anti-inflammatory drugs (NSAIDs) such as COX-2 inhibitors, were shown to be correlated with a reduction in long-term CRC incidence [112] and the risk of recurrence in randomized trials and meta-analyses [113]. Cole et al. presented an elegant meta-analysis of a randomized trial on the role of aspirin in chemoprevention [114]. With almost 2700 patients recruited in various clinical trials, treated with different doses of aspirin (low dose i.e. 80–160 mg/day or high dose, i.e. 300–325 mg/day), the result was a reduced risk of recurrence of colorectal adenomas, although this was mostly observed in the initial period of aspirin treatment.

Similarly, a double-blind trial to determine the effect of aspirin on the incidence of colorectal adenomas in over 600 patients with previous CRC confirmed that daily use of high dose of aspirin (325 mg/day) was associated with a significant reduction of colorectal adenoma incidence [62]. However, more recent studies by Veettil *et al.* show that the use of low-dose aspirin (80–160 mg/day) in patients with previous history of CRC or adenomas reduced the risk of recurrence of intestinal adenomas, whereas high-dose aspirin (300–325 mg/day) did not [113]. A recently launched ASPirin Intervention for the REDuction of CRC risk (ASPIRED) trial (NCT02394769) designed to reveal the benefit if aspirin in CRC prevention as well as to investigate its mechanism of action is ongoing [61].

In 2016 the U.S. Preventive Services Task Force (USPSTF) made *recommendations about the effectiveness* of aspirin in individuals at adequately high cardiac risk [60]. In adults aged 50 to 69 years who are at increased cardiovascular disease risk, the benefits of aspirin use include prevention of nonfatal myocardial infarction and ischemic stroke and, with long-term use, reduced incidence of CRC. Aspirin use may also result in small to moderate harms, including gastro-intestinal bleeding and haemorrhagic stroke.

Different effects of aspirin dose on cancer prevention were tested in The Cancer Prevention Project 3 (CaPP3) program that aimed in finding the right dose of aspirin for patients diagnosed with a mismatch repair gene defect, the underlying cause of Lynch syndrome (NCT02497820). This hereditary disorder affects individuals who have a higher than normal chance of developing colorectal or endometrial cancer. The primary objective of this study was to determine whether the cancer preventive properties of enteric coated aspirin in Lynch syndrome are dose sensitive by comparing overall cumulative Lynch syndrome cancer incidence rates after 5 years in people who took 100 mg, 300 mg or 600 mg enteric coated aspirin for at least 2 years. The secondary objective is to compare overall cumulative incidence of primary colorectal cancers. The results of this randomised, double-blind trial are expected in 2020.

Summarizing, no single agent predictably or completely suppresses adenoma growth in overlall population. Newer trials are evaluating combinations of agents of differing mechanisms of action acting synergistically are being developed.

2.2. CRC treatment

2.2.1. In vitro studies

2.2.1.1. Amantadine and pleconaril. Human endogenous retroviruses (HERVs) are a footprint of ancestral germ-cell infections in which viruses integrated into the host genome [115]. HERVs were reported to be expressed in ovarian, testicular and placental tissue stem cells and were related to carcinogenesis [116]. *HERV-K* transcripts of the ENV protein, known to be absent in physiological breast tissue, were shown to be overexpressed in the majority of breast carcinomas [117]. The expression of other proteins such as HERV-H [118] and HERV-3-1 has previously been reported in CRC tissues [119,120]. Diaz-Carballo *et al.* tested the antiviral drugs, amantadine and pleconaril, which target HERV components expressed in chemotherapy-naïve and chemoresistant CRC cell lines [63]. The mechanism of action of both medications is still poorly understood.

Amantadine, which acts on the viral M2 protein, thus hindering virus uncoating once it is taken inside the cell by endocytosis, was previously reported to suppress proliferation and to induce apoptosis in hepatocellular carcinoma cells [121]. Pleconaril integrates into the capsid of picornaviruses, preventing the virus from attaching to cellular receptors and preventing RNA release into the cell [122]. Both amantadine and pleconaril as monotherapies, in combination, or when combined with classic chemotherapy (doxorubicin, 5-FU or cis-Pt) reduced CRC cell viability [63]. Interestingly, in the chemoresistant $HCT8^{RETO}$ cell line, amantadine interacted with doxorubicin, 5-FU or cis-platinum (cis-Pt) in a synergistic manner. Therefore, there is some preliminary evidence that elevated expression of HERV proteins after cytostatic treatment might highlight potential therapeutic targets for CRC treatment. *In vivo* data are needed to confirm results obtained *in vitro*.

2.2.1.2. Disulfiram. Example of a mCRC-proposed treatment includes disulfiram, an FDA-approved medication initially for treatment of alcoholism. Based on promising *in vitro* results obtained in combination with copper in a panel of naïve, oxaliplatin- or irinotecan-resistant human CRC cell lines [64], a phase II clinical trial in mCRC patients who failed oxaliplatin or irinotecan treatment is currently planned [64]. The recent study of Skrott *et al.* revealed that the molecular target of disulfiram responsible for its anti-cancer activity is an adaptor of human ubiquitin selective protein segregase p97, known to play a role in turnover of proteins orchestrating stress-related signaling pathways [65,123].

2.2.2. In vivo studies

2.2.2.1. Ivermectin. Based on the documented involvement of canonical WNT-TCF signaling pathway in many complex diseases, including CRC, Melotti *et al.* preformed a repositioning screen for WNT-TCF response blockers [124]. Using a transcriptional reporter assay for TCF activity, tracking the genetic changes afforded by dominant-negative TCF used as a benchmark, and a Microsource 1040 small molecule library of clinically tested small molecules, the anti-parasitic drug ivermectin [125,126] and related macrocyclic lactones of the same drug family were identified as effective WNT-TCF response blockers in colon and lung cancer cells. Their findings were validated in CRC preclinical models of tumor growth with cell-line and patient-derived primary tumors. Mechanistically, ivermectin appears to indirectly alter the levels of C-terminally phosphorylated β-CATENIN forms, leading to a decrease WNT-TCF signaling transcriptional response. As with most if not all drugs, ivermectin has multiple modes of action and interacts with different macromolecules in different contexts. For instance, it is a very well-known anti-helmintic drug that activates glutamate-gated chloride channels thus altering chloride influx and preventing neural excitation, leading to bug paralysis and death [127].

The off-patent status of this drug and the knowledge on safety and dosages offers ample opportunity for affordable approaches and for novel formulations for specific WNT signaling response-dependent indications notably including cancers of the skin, breast, lung, ovary and colon. Nevertheless, care should be taken to avoid passage of the drug through the blood brain barrier (e.g. [128,129]). Similarly, the increase in metastases reported in xenografts after blockade of TCF responses [66,80,81] indicates the need to find the right therapeutic pharmacological window to inhibit local tumor growth without enhancing metastases. It could also be re-positioned to use against already established metastases that may again be WNT-dependent after surgical removal of all primaries, or even for CRC prevention. Moreover, there are multiple cancer indications that are WNT-dependent and against which ivermectin could be quickly adopted.

Another important parameter to consider is so called “therapeutic index” (TI) that represents a dose range at which a drug is effective without adverse effects. In clinical surroundings, the TI is the range of doses at which a drug appears to be effective in clinical trials for a median of participants without unacceptable side effects [130]. The USA FDA defines a narrow therapeutic index drug (NTID) when (i) there is less than a two-fold difference in median lethal dose (LD_{50}) and median effective dose values (ED_{50}), (ii) there is less than a two-fold difference in the minimum toxic concentrations and minimum effective concentrations in the blood and (iii) safe and effective use of the drug requires careful titration and patient monitoring [131]. There are multiple examples of NTIDs, i.e. drugs for which the difference between dose range or blood concentrations that leads to efficacy and that induces side effects is very small [130], e.g. carbamazepine, cyclosporines or digoxins at all oral dosage forms. TI for digoxins is between 2 and 3, i.e. doubling or tripling the recommended dose would lead to toxicity [132].

Due to the fact that ivermectin, a substrate of P-glycoprotein (P-gp), belongs to the NTID class, defective P-gp function can cause serious adverse drug reactions due to increased brain penetration and/or decreased clearance [133]. It is therefore important to ensure that (i) benefits of treatment with a drug candidate would outperform the side effects and (ii) careful titration and PD monitoring is necessary to obtain desired clinical outcome [134].

2.2.2.2. Diclofenac. An inhibitor of COX-2 and prostaglandin E2 synthesis, Diclofenac displays pleiotropic effects in various processes such as angiogenesis, the immune system, tumor metabolism or radio- and chemo-sensitivity. It was originally used as an anti-pain medication in rheumatoid arthritis, treatment for actinic keratosis and other musculoskeletal conditions, migraine, fever, acute gout or post-operative pain [68]. It is not available as a generic drug. Notably, initial support for the use of diclofenac in CRC treatment derives from experimental animal models [67–69]. Treatment of murine colon adenocarcinoma C-26 tumors with Diclofenac resulted in apoptotic cell dead and dose-dependent tumor growth inhibition [67]. In the same tumor model Diclofenac applied in combination with hyaluronan induced tumor growth inhibition accompanied with tumor anti-angiogenic effect, increased apoptosis and tumor necrosis [135].

Diclofenac is a component of a drug combination mixture that has proven efficacious against in a CRC mouse model with liver metastases containing in addition to DFC, cimetidine, low dose cyclophosphamide and sulfasalazine. CB6F1 mice bearing CRC liver metastases treated with the drug combination showed significant tumor growth delay, inhibition of tumor angiogenesis and induction of tumor necrosis, with both partial and complete remissions recorded. Moreover, overall survival in both partial and complete remission groups was significantly longer than untreated controls or animals treated with rapamycin (an mTOR inhibitor) and anti-VEGF B20 antibody separately, used as positive controls [70]. Importantly, the use of this drug combination significantly hampered hepatic metastasis perfusion proving the therapeutic activity in treatment of challenging advanced metastatic stages of CRC.

2.2.2.3. Captopril, irbesartan and TCV116. There is increasing evidence that the risk of CRC can be approximately 30% higher in patients diagnosed with type II diabetes than in non-diabetic patients [136]. The association between these two disorders is recognized by the American Diabetes Association since 2010 [137] and can be seen in shared risk factors, epigenetic changes or cellular and molecular pathways, e.g. activation of inflammation and WNT/β-CATENIN pathways [136]. Moreover, the Genome-Wide Association Studies (GWAS) indicated certain genes, previously liked to diabetes, which may very well contribute also to the CRC development [138].

CRC cells express Renin and Chymase through which, in the hyperglycaemic conditions, can activate a hypertensive hormone, Angiotensin. Angiotensinogen is a liver-derived hormone and a component of the Renin-Angiotensin system (RAS) that regulates blood pressure and fluid balance. CRC cells that activate Angiotensin metastasize preferably to the liver [139]. Diabetic patients that present high activity of Rennin and Angiotensin-II (A-II), an active form of Angiotensin, in primary tumors, show faster disease progression and liver metastasis [139]. The RAS-activating system is a known molecular target for CRC prevention and treatment. Angiotensin-II is known to be a pro-tumoral factor, which leads to vasoconstriction and induction of various growth factors, proliferation in neoplastic tissues and metastasis progression [140]. Therefore, treatment with hypoglycaemic agents together with anti-angiotensin agents might be a potential strategy to inhibit CRC metastasis.

Indeed, in a diabetic mouse model, liver metastases of murine CRC CT26 cells were suppressed by anti-angiotensin treatment combined with a Chymase inhibitor, a Renin inhibitor, and an A-II receptor blocker. Moreover, concurrent treatment with hypoglycaemic and anti-angiotensin drugs resulted in a synergistic inhibitory effect on liver metastasis and prolonged survival as compared to hypoglycaemic drugs alone [72]. Furthermore, blocking of RAS using captopril has been suggested not only to inhibit tumor growth but also to improve liver regeneration in mice experiencing partial nephrectomy [71].

Angiotensin converting enzyme inhibitors (ACE-I), as well as Angiotensin II type 1 receptor (AT1R) antagonists were reported to display anti-cancer activity via anti-angiogenic mechanism of action [73]. AT1R expression showed a direct correlation with tumor stage and liver metastasis in CRC patients [141]. AT1R blockade by AT1R antagonists might therefore inhibit carcinogenesis or angiogenesis efficiently [73]. Using this approach captopril (an ACE-I, administered daily via intraperitoneal injection) and irbesartan (an AT1R antagonist, administered daily via subcutaneous injection) were tested in a murine mCRC model. The joint treatment significantly reduced the number of liver metastases via impairment of tumor vascularization. However, it did not influence the overall survival of treated animals [73]. Another AT1R antagonist, TCV116 (candesartan), when administered orally, showed preventive effects in tumor metastases in a mouse renal cancer lung metastases model, in which anti-angiogenic mechanisms were predominantly involved [75].

2.2.2.4. Mesalamine. Mesalamine, also knowns as Mesalazine or 5-aminosalicylic acid is a weak COX and lipoxygenase (LOX) inhibitor, structurally related to NSAIDs, but unlike the latter it has low systemic resorption and very few side effects, even at high doses during long-term use [142]. In several studies mesalamine was reported to decrease the growth and survival of CRC cells via various signaling pathways [143,144]. For example, Bos et al. showed that mesalamine inhibition of the WNT/β-CATENIN pathway is dependent on increased N-terminal phosphorylation of β-CATENIN [145]. Moreover, Fina *et al.* reported that mesalamine enhanced CRC cells anoikis, a form of programmed cell death triggered by the loss of anchorage to the extracellular matrix [143,144]. Although definitive conclusions on anti-CRC activity of this drug cannot be yet drawn, the existing data seem to indicate that it can interfere with colon carcinogenesis [146]. The recently opened phase II multicenter, multinational, randomized clinical trial (NCT03070574) with a primary objective to investigate the effect of regular treatment with mesalamine on the occurrence of colonic neoplasia, tumor multiplicity (the number of detected adenomas/carcinomas) and tumor progression in Lynch Syndrome patients (with the exclusion of participants with regular use of NSAIDs or COX-2 inhibitors) should resolve the potential benefit of mesalamine treatment.

2.2.2.5. Cimetidine. H_2-histamine receptor antagonists, such as Cimetidine, act by blocking the action of histamine on gastric parietal cells, which in turn leads to the reduction of gastric acid [147]. Interestingly, Cimetidine also inhibits the expression of E-Selectin, one of the key molecules in the vasculature to which cancer cells adhere via interaction of its ligand. This anti-adhesive feature of cimetidine [148] might be seen as an additional mechanism of action against tumor metastases. Multiple studies described the role of adhesion molecules, including selectins, that are involved in inflammatory process are also implicated in the immune response during the trauma such as a major surgery [149]. Interestingly, Cimetidine used as peri-operative adjuvant CRC therapy was found to reduce post-operative immunosuppression [78] with a considerable higher 3-year survival as compared to the control group [79]. This peri-operative action of cimetidine was especially favorable in tumors that overexpress sialyl Lewis-A antigen (sL^A) and sialyl Lewis-X (sL^X) antigen, mostly in the gastric and colorectal regions in patients diagnosed with CRC Dukes C stage and patients with non-resectable CRC [150]. 10-year overall survival was significantly longer in patients where cancer cell expressed high levels of sL^A and sL^X. On the other hand, the beneficial use of cimetidine in terms of prolonged overall survival was not observed in patients with low levels of sL^A and sL^X [150].

2.2.2.6. Acetylsalicylic acid (Aspirin™). Many studies have shown that regular use of aspirin after CRC diagnosis is associated with a superior clinical outcome [151,152]. It is known that inhibition of prostaglandin-endoperoxide synthase 2, known as cyclooxygenase-2 (COX-2), by aspirin downregulates phosphatidylinositol 3-kinase (PI3K) signaling activity, which plays a major role in carcinogenesis [153]. Interestingly, Liao *et al.* showed that the use of aspirin was associated with longer survival among patients with mutated-PIK3CA colorectal cancer, but not among patients with wild-type PIK3CA cancer [154]. Sensitive cancers could thus be addicted to PIK3CA activity and ensuing AKT function. The activity of aspirin seems to counterbalance the enhanced cancer cell growth and enhanced activity of downstream events linked to mutated-PIK3CA, possibly including stemness pathways such as non-canonical GLI signaling [155,156]. Further studies are necessary to fully confirm these observations.

In addition to the association of low-dose aspirin with a reduction in CRC incidence, there is also growing evidence suggesting that this common drug, used as an adjuvant treatment following the diagnosis of cancer, may reduce metastatic spread and may increase the survival of patients. In systematic comparisons of observational studies and randomized trials, the regular use of aspirin was associated with reduced risk of CRC, as well as 20-year risk of death due to CRC and with a decreased chance of distant metastases [157]. It was demonstrated that platelets support the recruitment of colon cancer cells from the bloodstream under physiological levels of shear flow[158]. Low-dose aspirin (also called anti-platelet doses, i.e. 75 mg/day corresponding to 15–20 μM of salicylic acid in plasma) prevent platelets from binding to tumor cells but also inhibit cancer cell proliferation through the inhibition of platelet-derived signals necessary for the upregulation of the oncoprotein c-MYC [158]. The latter was found to be activated and overexpressed in tumor cells mostly at the “metastatic niche”, a specific microenvironment where metastatic tumor cells would engraft, as compared to primary tumors [159]. Therefore, aspirin administered at anti-platelet doses may lead to metastasis inhibition and improved survival via the inhibition of platelet-derived signals necessary for the

upregulation of c-MYC [160,161].

One of the key pathways responsible for growth and differentiation of colonic cells is the WNT/βCATENIN (β CAT)/TCF signaling pathway. Mutations in the *APC* gene, which encodes a negative regulator of βCAT, initiate the development of almost all human colon cancers [162,163]. Aspirin was shown to attenuate transcription of a TCF-responsive reporter gene and to downregulate the WNT/TCF transcriptional target *CYCLIN D1* by modulating TCF activity without disrupting βCAT-TCF complex formation [164]. Therapeutic blocking of WNT-TCF signaling may not be straightforward: whereas it will likely inhibit intestinal tumor growth, there is evidence that it can also promote metastasis [66,80,81].

2.2.2.7. Epirubicin. It is worth mentioning epirubicin, a drug previously used in oncology. Over 20 years ago Plosker *et al.* showed that a fraction of mCRC patients resistant to oxaliplatin exhibited *DNA TOPOISOMERASE 2-α* (*TOP2A*) gene amplification [165]. TOP2α plays a key role in maintaining the topological status of chromosomes during DNA replication and transcription. Epirubicin, a 4' epimer of the anthracycline antibiotic doxorubicin, actually targets TOP2α and thus interferes with the synthesis of DNA through intercalation, being most active in the S-phase of the cell cycle [165]. Epirubicin has been used in a variety of malignancies as monotherapy or in combination with other cytotoxic agents. Currently, there is an ongoing open-label, single-arm, phase II study with epirubicin in patients with oxaliplatin-refractory mCRC. Should epirubicin prove its efficacy with the primary end-point of progression-free survival in patients with *TOP2A* amplification, it could become a novel treatment for this patient subpopulation [82].

2.2.2.8. Metformin. Repurposing anti-diabetic drugs in mCRC relies on the finding that the microenvironment of diabetes, such as hyperlipidemia, local inflammation/oxidative stress, altered microbiota and/or ischemia can promote the development of cancer [136]. Whereas the American Diabetes Association did not provide recommendations on the choice of anti-diabetic treatment in CRC [166], the initial anti-diabetic agent, metformin, has been associated with decreased incidence or better outcomes and prolonged survival in CRC patients [84,85].

Metformin, a biguanide derivative used to treat type II diabetes [167] and polycystic ovary- and metabolic syndrome [168] acts through the reduction of basal glucose output via the suppression gluconeogenesis and glycogenolysis in the liver, as well as increase glucose uptake by muscle. It is important to note that due to the fact that metformin does not directly stimulate insulin secretion and activates the duodenal adenosine monophosphate-activated protein kinase (Ampk)-dependent pathway [167], its activity is associated with a lower risk of side effects, such as hypoglycemia, than other oral anti-diabetic drugs. Mechanisms responsible for the antitumor effect of metformin include the reduction of the level of circulating insulin and the activation of AMPK [169].

Reports of the activity of metformin in mCRC patients, however, are not consistent. On the one hand, metformin was reported to be associated with enhanced overall survival (OS) and progression free survival (PFS) in mCRC patients with type II diabetes [170]. Mesalamine (see above) in combination with metformin induced synergistic CRC cell death through increases in oxidative stress, apoptosis [76] or mitotic arrest [143]. On the other hand, a phase II trial of metformin and fluorouracil in mCRC patients refractory to standard treatment, showed only a modest antitumor activity [56]. Additional clinical trials are thus necessary to confirm the activity of metformin for this indication.

2.2.2.9. Lifestyle. We wish to highlight the non-negligible importance of diet (the foremost source of external polypharmacological agents), psychological stress and lifestyle including exercise (which activates the endogenous production of multiple components with polypharmacological effects) in CRC prevention. This has been elegantly reviewed by Chan and Giovannucci [171] and by Strum [51]. The use of medications for disease prevention is hugely affected by such associated factors that can potentiate or significantly limit the utility of the drugs. Furthermore, other medical conditions such as obesity and diabetes mellitus [172-173] were clinically confirmed to be associated with an increased incidence of CRC in adults. A large-scale prospective cohort study with 680 cases of colon cancer and 330 Japanese patients (male and female) of rectal cancer was performed to confirm the association between perceived stress and colorectal cancer incidence with a 21-year follow-up [174]. The results analysis revealed a significant association of perceived stress with rectal cancer incidence but not with colon cancer incidence. An earlier study reported on the correlation of stress *intensity* and frequency with risk of rectal cancer in Danish population [175]. A high level of perceived stress was significantly associated with the risk of rectal cancer only in men, but no other significant associations were found for colon or rectal cancer in women. On the contrary, frequent stress was significantly associated with the risk of colon cancer only in women, not in men. However, this study did not prove the connection between perceived stress itself and the risk of colorectal cancer. The differences between both above-cited studies might be due to geographical, genetic and incidence differences between the tested populations. Additional meta-analyses of data pooled from 12 European cohort studies including over sixteen thousand men and women aged between 17 and 70 years of age, who were free from cancer at the study baseline and were followed-up for a median of 12 years, revealed that work-related stress is unlikely to be a significant risk factor not only for colorectal, but also lung, breast, or prostate cancers [176]. It should be noted that due to the fact that there is no simple dose-response relationship between stress and cancer incidence, establishing an association between psychological stress and cancer incidence might be very difficult [177]. In general, while exciting and eventually most useful, clear prevention data from lifestyle may be hard to obtain for many indications, given the large cohorts required, the many variables and the long times involved [178,179].

In summary, a number of repurposed drug candidates has already been evaluated preclinically or clinically for CRC prevention or treatment. The above-listed analysis of the agents reveals NSAID pharmacological class as the most studied agents for prevention and treatment of various stages of CRC. Many of them represent untapped potentials alone or in combination that needs to be further investigated, especially since the number of off-patent drug candidates is increasing. In addition, it is critical to start a 'personalized repurposing' approach. To do so, we need to effectively and quickly combine massive pharmacological, genetic, omics, clinical and other data, prompting the development of bioinformatics tools to cope better with the complexity of biological systems and speed up drug development and patient treatment.

3. Computational approaches in drug repurposing

Beyond prevention, dealing with the increased incidence and mortality of CRC requires novel approaches to find new and effective drugs. With the rapidly growing evidence for tumor heterogeneity and the identification of key driver pathways through genomics, it is possible now to begin to link gene-expression profiling data with screens for drug repurposing. Moreover, systems biology approaches combined with pharmacology are being currently used to identify new beneficial off-targets that can be used for efficient drug repositioning [180]. Combining both pharmacokinetic and pharmacodynamic network analyses, together with genome polymorphisms may lead to promising new therapeutic strategies [181]. It is important to note, however, that a careful selection of pertinent and predictive preclinical models to be used for the *in vivo* evaluation of drug candidates remains essential.

Below we present the emerging computational approaches based on transcriptomics, genomics, side effects, phenotypes or their combinations. Even if not reported yet for CRC treatment, they seem to be the

promising tools to be further exploited. Other omics approaches, such as proteomic, epigenomic and metabolomic profiling, are not specifically discussed but fall within the possibilities included in the overall pipeline scheme.

3.1. Text-mining approaches

Literature-based discovery (LBD) or text-mining is a possible strategy to look for new indications of existing drugs. It is not entirely easy to find often hidden connections between biomedical entities. Together with an immense development of MEDLINE, PUBMED and other large-scale databases, several techniques have been developed in order to extract biomedical terms and their interrelations from the scientific literature and help to generate new hypotheses [182]. Text-mining technologies provide a solution bridge the knowledge gap between free-text and structured representation of related information in cancer research. Another issue is to what extent systems that rely on knowledge and computational methods can convert text data into useful clinical information [183,184]. One way to organize existing knowledge on cancer is to utilize Hallmarks of Cancer, i.e. normal cell characteristics (i.e. hallmarks) to behave as malignant cells. Those include sustained proliferative signaling, induced angiogenesis, evaded growth suppressors, resistance of cell death, enabled replicative immortality, and activation of invasion and metastasis [185].

Baker *et al.* introduced hallmarks of cancer taxonomy and developed a supervised machine-learning tool called Cancer Hallmarks Analytics Tool (CHAT: http://chat.lionproject.net) that is capable of retrieving and organizing millions of cancer-related references from PubMed into the taxonomy [184,186]. CHAT classifies over 150 million sentences extracted from over 24 million PubMed abstracts. This approach collected more documents and identified lower percentage of false positives than any regular keyword-based search. Following this research, ontology tools were developed, e.g. OncoCL [187] or OncoSearch [188], harvesting interactions between genes and proteins, as well as relations between environment and cancer from high-throughput omics data and scientific literature.

Other text-mining tools were developed to extract side effects data from clinical trials (ClinicalTrials.gov) and identify the drugs with fewer events in the context of selected disease [189]. Additional text-mining approaches are dedicated to searching other types of repositories, e.g. chemical databases. This approach called DrugQuest (http://bioinformatics.med.uoc.gr/drugquest) was designed to cluster DrugBank records based on text attributes in order to find new associations between drugs. [190]. Novel analytical tools are increasingly at the forefront of repurposing, with bioinformaticians often relying on text-mining approaches to find connections between drugs and diseases. One of the examples of successful use of text-mining techniques for drug repurposing in CRC is the Repurposing Drugs in Oncology (ReDO) project designed to rapidly identify new and effective cancer treatments characterized with low toxicity that are cost effective [68]. They successfully used a literature-based approach using all forms of published data to find compounds to repurpose [68].

3.2. Transcriptomics

The data on all the RNA molecules of a cell or groups of cells, or transcriptomic data, can identify statistically significant differentially expressed genes, for instance over- and underexpressed genes in (i) disease conditions vs. normal or (ii) drug-treatment groups vs. control. Such lists can subsequently be used to evaluate pathways or networks that might be disregulated. Using systems pharmacology methods, a huge collection of genome-wide gene-expression readouts of cell lines treated with more than 1000 drug-like chemicals was created and is available as the Connectivity Map (CMap) [191]. CMap provides a transcriptomic platform for large-scale pattern-matching strategy to identify the differences or similarities in genomic signatures among complex disorders, functional gene sets, and medications. It provides a starting point for drug repositioning based on expression profile similarity. CMap has been widely used for discovering of repositioned medications of complex diseases such as diabetes, Alzheimer's disease or cancer.

The CMap was also used to identify the connections between the drugs themselves to suggest the repositioning. Over 1300 molecules in CMap were connected into a network using MANTRA (Mode of Action by NeTwoRk Analysis, http://mantra.tigem.it). These therapeutics were partitioned into groups by similarity in mode of actions or acting on exactly the same signaling [192]. Such an approach grouped together compounds interacting with distinct members of the same pathway therefore enabling to find previously unrecognized modes of actions of well-characterized drugs by simply looking for the drugs neighbouring a drug of interest.

Due to the fact that the most of CMap compounds are FDA-approved drugs, it is becoming a powerful tool for drug repositioning. CMap was based on (i) comparison of a set of different gene expression microarrays (a set of control and a set of a patient), followed by (ii) the creation of a score of the match between the set of differentially expressed gene and genomic profiles of drugs given by differentially expressed genes, and (iii) ranking the medications by a score. CMap was based on the observation that drugs with a similar mechanism of action *in vivo* also often elicit similar expression responses in this *in vitro* system, thus a drawback of this method is that no specific references to any biological functions associated with the disease are considered.

To address this problem, the Functional Module Connectivity Map (FMCM) for the discovery of repurposed drug compounds to treatment complex disorders was created in 2014 [86]. “The FMCM is based on condition-specific function-function networks (FFNs) and applied a gene-selection-by-trend-of-progression procedure (GSToP) to identify complexly connected and highly expressed hub genes in the FFNs” claim the authors [193]. As a result, the authors identified agents having high inhibitory activities against cancer cells. Moreover, each therapeutic in the component drug was beneficial to all the functional modules while none of component drug was detrimental to any of the modules. The identified drugs were (i) anti-hypertention dibenzyline phenoxybenzamine, (ii) cyclin-dependent kinase inhibitor GW-8510, and (iii) antibiotic Primaxin [86] and indeed showed high activity against cancer cells.

Interestingly, Hsieh *et al.* reported recently the potential application of GW-8510 for the treatment of CRC underlying the importance of human ribonucleotide reductase 2 (RRM2) blockade [87]. These *in vitro* findings need further validation and are limited by the availability of gene expression profiles of drugs in the databases used at the publication time. Nevertheless, FMCM seems to be a promising tool in drug repurposing for CRC, but also other tumor types or complex conditions.

The next level of transcriptomic data to be used will likely derive from single cell RNA sequencing, which nowadays can allow the identification of transcriptomes of several million cells simultaneously (e.g. [194]. This approach will be key to understand and manage the heterogeneity within and among cancers as well as interactions of the tumor cells with the surrounding stroma and immune system.

3.3. Genetics and genomics

Human genetics has established different lines of evidence linking specific genes and specific diseases. Genome-Wide Association Studies (GWAS) have allowed a potential drug to undergo repositioning to another indication if its known protein target is genetically associated with a disease that is not among those for which the drug is used. This tool was used to identify the CRC-related common low-penetrance susceptibility loci in certain patient populations, e.g. European [195], multicontinental [196], or Asian [197], East Asian [198]. More integrated studies combining data of over 52,000 patients from large CRC consortia, i.e. the Colorectal Cancer Transdisciplinary (CORECT) study,

the Colon Cancer Family Registry (CFR), the Molecular Epidemiology of Colorectal Cancer (MECC) study, and the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) revealed six new susceptibility risk regions for CRC at loci 3p14.1, 3p22.1, 10q24.2, 12q24.12, 12q24.22 and 20q13.13 [138].

Implementing the reverse gene-expression profiling method, Van Noorth *et al.* compared non-metastatic tumors vs. metastatic primary tumors and metastases in order to define a gene signature of metastatic potential [199]. Contrary to other reported methods, where diseased states were compared to healthy tissues (e.g. [200]), the authors used patient's primary non-metastatic tumors vs. tumors of various grades, as well as metastases, to compare gene profiles. Their CMap-based computational prediction revealed the anti-diabetic drug troglitazone, the anti-fungal enilconazole, and the anti-depressant citalopram as the most promising drug candidates for *in vivo* validation. These compounds were active via the inhibition of TGFβ signaling but citalopram was the best-tolerated and was tested for metastasis inhibition in an orthotopic xenograft model [88]. The number of liver metastases, as well as the number of circulating tumors cells were reduced as compared to the control group. The primary tumor size was significantly inhibited by citalopram, which is good news but makes an interpretation of the ability of the drug to act directly on metastases difficult. Nevertheless, this study confirms the general usefulness of the approach. It could be further developed, for example by including additional drug side effect data that could help in the elimination of potentially toxic drugs identified via gene profiling.

Overall, it is important to note that CMap can be widely applied to any potential drug although its prediction power remains to be validated at large scale. In addition, the use of gene expression approaches involves presently certain weaknesses, such as (i) profiles generated under uncoordinated conditions can lead to low-precision predictions and (ii) no information on the drug-disease or drug-side effects are included in the gene expression data [201].

3.4. Side effects and phenotypes

Although over the years genomics and transcriptomics-driven methods have suggested novel oncogenic driver mutations, many remain untested but could be supported (or not) by patient-specific responses to targeted treatments. This is generally difficult to achieve due to the lack of (i) specific drugs for each potential driver, (ii) reliable biomarker-driven treatment strategies, (iii) reliable patient-specific and clinically relevant in vivo preclinical models, (iv) detailed information on all diseases, (v) homogeneity of CRC [202].

Each time a doctor examines and diagnoses a patient, he/she relies on the phenotypic characteristics and symptoms of that patient. According to the definition the phenotype can be defined as *the observable physical properties of an organism; these include the organism's appearance, development, and behavior* [203]. Thus, the urgent need for affordable personalized medicine becomes even more clear in cases of cancer. On the upbeat side, phenotypically-driven screens are routinely performed in the drug-discovery settings, leading to clinical drug approvals [204]. Rigorous *in vivo* preclinical validation is thus essential, even if the phenotypes that are (or can be) scored in preclinical animal models are not always perfectly medically relevant or predictive.

In this context, the effort has always been to track direct, positive effects on disease betterment. However, any drug will have multiple effects at different doses. In this context, surprisingly, the side effects of or adverse drug reaction (ADR) to a medication can potentially serve as "phenotypic biomarkers" for disease treatment. As mentioned in the introduction, the current use of sildenafil or exenatide is based on robust clinically observed side effects (penis erection) independent of the original indication (angina and diabetes type II, respectively). In some cases the side effects or ADRs are caused by changes of the intended drug's primary target (which might for instance not be expressed in the tissue showing side effects, this tissue could have higher expression of a lower affinity target, etc.), whereas others can result from non-specific interactions of reactive metabolites [205,206] not taken into consideration during preclinical development.

Compiled ADR data were used to build a large-scale, prospective evaluation of safety target prediction called the Similarity Ensemble Approach (SEA) [207]. The SEA relies on the chemical similarity of drugs and can be used for targets with already known ligands [208]. Lounkine *et al.* developed a computational approach to predict the activity of 656 marketed drugs on 73 "side effect" off-targets with known ADR. Since the majority of predictions were validated in *in vitro* binding assays, a drug-target-side-effect network was created that linked the new targets to the drug's side effects. This lead to the prediction of new, primary targets. Even if this method still requires experimental testing *in vivo*, it can significantly help to prioritize off-targets for clinically use.

Yang and Argawal used the public SIDER database as a source of compiled side effects in order to link diseases and drug repositioning possibilities [209]. Individual drugs connected to specific pathologies were extracted using pharmacogenomics data available on the Pharmacogenomics Knowledgebase (PharmGKB) [210]. Moreover, the side effects based on the clinical evidence were taken from the drug information sheet. Using this combined strategy, the authors annotated over 3000 side effects-disease relationships that can lead to drug repurposing. Importantly, the authors showed that the drugs used to treat similar disorders cause similar side effects. In other words, on the one hand side effects could serve as an indicator of a certain mode of action, and on the other, the drugs with the same side-effects might be potentially applied to threat the same disorder. The authors went on to predict new drug indications although there was no experimental validation reported. This study underlines the importance of clinical side effects ("clinical phenotypic assay) and their potential use in drug repurposing.

Given that the number of side effects (defined as effects apart from the sought-after targeted action) per drug in the human body may approach seventy on average [211], the data analysis to understand each effect could be a daunting. However, computational approaches could utilize specific recorded drug action side effects to generate new possible indications. Indeed, side-effects observed in clinical phases of drug trials were exploited as a potential source of phenotypic information [212]. Campillos *et al.* used phenotypic side-effect similarities to infer whether two drugs might share a target. Applied to over 700 marketed drugs, a network of 1018 side effect-driven drug-drug interactions was developed, 261 of which were formed by chemically distinct drugs from completely different therapeutic indications. Thus, the network of drugs, with at least 25% of probability of sharing a target was created. The predicted drug-drug relations were subsequently tested experimentally and drug-target relations ware validated in *in vitro* binding assays.

All validated predictions shared a target with a drug from a different indication area.

As mentioned above, while some side effects originate by the modulation of the primary target or by reaction with reactive metabolites, in many cases, ARS are caused by unintentional activity on off-targets. Therefore, the predictions of off-target-drug interactions may be useful for the prioritization of drug candidates for further development.

It is noteworthy that the presence of side effects depends not only on the doses of a particular drug but on many other factors such as the time of exposure, age, genetic variation and epigenetic states. Furthermore, the frequencies of observed side effects in the whole population may underestimate the importance of a given side effect observed on any given patient. Moreover, some side effects may be non-specifically induced by a medication of interest, or by its degradation by-products [209].

Therefore, the inclusion of (i) multiple factors that actually determine or contribute to the side effects, (ii) identified side effects in a particular patient and (iii) the pharmacologic data of individual

patients and the groups should be used together to define phenotypic/cause profiles of a drug that could further serve for additional disorder indications and thus to repositioning possibilities.

3.5. Integrated approaches

Whereas different data sources may provide complementary information, such as genomics or phenotype, their integration will give better results. Moreover, since the association of accurate indications with drug candidates (for both FDA approved and experimental compounds) in a wide range of diseases requires very large-scale matching. The current attempts of merging different approaches at such scales consist of systematic analyses of pharmacokinetics, pharmacology and/or systems biology via merged computational and experimental approaches. In this sense, a number of machine-learning methods have been developed in recent years to predict drug-drug interactions and novel drug indications in a time- and cost-effective way.

3.5.1. Differential evolution

An interesting strategy for designing new treatments is based on the linking of machine-learning with phenotypic data. A number of top-down approaches treat a system (e.g. a cancer cell) as a “black box” and link the system response (output) to a treatment (input). Those techniques help predict the output of the system for a given set of input constrains without unravelling the details of the sequence of events inside the system, i.e. without resolving the exact mechanism of action in the cancer cell [213,214]. Therefore, instead of trying to understand all the complexity of molecule-to-molecule interactions in the system of interest, e.g. the molecular mechanism responsible for observed activity in a particular cancer cell (phenotype) or, worse, in a heterogeneous tumor that quickly evolves, these approaches search in the first instance for the optimal system response under a given input, averaging out cancer cell heterogeneity. Cleanly defining the output or response phenotype is thus essential for this approach to work.

An accurate example of such a methodology is the Feedback System Control (FSC), that is based on a machine-learning strategy called Differential Evolution [215]. FSC may not only analyze the input-output relations, but also guide the complex system to the desired endpoint (e.g. cancer cell death) without knowing the intersystem connectivity between molecular components. FSC is an artificial intelligence-based method [216]. FSC selectively samples a minimal number of experimental data points in order to create the cell’s response surface to drug combinations in terms of second-order linear regression models that are used to select the optimal treatment. It is important to note that in contrast to other approaches based on pharmacogenetics or high-throughput screening, this approach identifies suggested optimal treatments with minimal experimental effort, thus reducing significantly the time and the cost of the ‘primary’ screen. FSC deals with the drug-dose relation of drug combination, as compared to monotherapies, and can be used to screen for ‘optimal side effects’ of for new indications with FDA-and EMA-approved drugs. Moreover, this method does not limit the search to synergistic drug pairs, but offers the possibility of the identification of multi-drug combinations.

The FSC platform has been successfully applied to navigate the experimental parametric space of nine angiostatic drugs [217,218] and ten anti-cancer compounds [219] applied at low-doses. In all cases, the rapid, iterative approach of *in vitro* testing for cell viability (using CellTiterGlo™ luminescent cell viability protocol with the ATP level measurement as a readout), together with second-order linear regression modeling, allowed for the identification of an optimal, synergistic, low-dose drug combination. This tool is currently being used for drug repurposing studies and the results are pending. Similarly, *in vivo* testing with PDXs is expected to confirm, or not, the predictions from the FSC model.

3.5.2. Drug-drug and disease-disease similarity measurements

Following the development of CMap (see above), a third type of approach was developed consisting of a method for PREdicting Drug IndiCaTions (PREDICT) using drug-drug and disease-disease similarity measurements [220]. The strength of this approach was based on the fact that it can operate on marketed drugs with approved uses, as well as on new drugs with yet undefined indications. The authors applied a logic regression scheme in order to measure and rank the similarity of drug and disease to drug-disease pairs of a known association. The prediction was performed using a drug-disease association data set established by the authors together with a set of novel drug-drug similarity data. The prediction results were subsequently ranked using the score cut-off with the best statistically significant values against a drug indication. Top-ranked predictions were then validated against indications tested at the clinical level, as well as with the co-occurrence of drug targets and indicated diseases in the same tissues. The similarity measures were multifactorial and contained drug-drug similarities that in turn were based on (i) chemical fingerprints, (ii) side-effects, or (iii) sequence alignment score [221] between the corresponding drug and related genes. In order to give to the study a personalized medicine twist, the authors replaced the disease-disease connotations based on phenotypic similarity with patient gene expression profiles. Thus, PREDICT could be potentially used to identify the optimal drug to each patient. The analysis of the same input information on disease genetic signatures and drugs (such as chemical structures or side effects) with PREDICT resulted in a high performance evaluation as measured by an area under the Receiver-Operating Characteristic Curve (AUC) score. This score of a given drug–disease association (d_r, d_i) is calculated by considering the similarity, for the given pair, of all known drug–disease associations to this association. For each known associations the drug–drug similarity $S(d_r, d_r')$ and the disease–disease similarity $S(d_i, d_i')$ were calculated. In the next step the two similarities were combined into a single score by computing their unweighted geometric mean. AUC score value for PREDICT had a high value of 0.92 ± 0.02, whereas when CMap was used, which predicts drug–disease associations by looking for drug response gene expression profiles that anti-correlate with a disease signature, the AUC score was relatively low (0.45 with no standard deviation given).

3.5.3. Gene-chemical structure-target networks

Two years later Napolitano *et al.* used a similar approach with a different machine-learning algorithm [222]. The authors aimed to predict the therapeutic class of FDA-approved compounds, focusing primary on drug characteristics and not considering diseases-related information. The reasoning behind this choice on this fourth approach discussed in this review was motivated by the fact that the currently available data on diseases are sparse and highly variable. Moreover, they are often derived from patients that were already pre-treated with other medications, inducing another level of complexity in data interpretation. Their machine-learning algorithm used the discrepancy between the predicted and known drug classification as a potential alternative indication. In the computational pipeline various data sources were included such as (i) CMap gene expression analysis of over 1200 drugs, (ii) approximately 6600 drug chemical structures and (iii) over 1500 drug targets. For each data source pairwise similarities were evaluated, e.g. drug-drug similarities for the gene expression were performed. This was done based on the ranking of the genes in every drug-induced expression profile and subsequent statistical importance analysis (p-values). In the next step, pairwise similarities for each molecular structure by calculating the interval between the binary fingerprint of interest. Lastly, target-based similarities were added together with their distances across the global human protein-protein interaction network. All the drug similarity information was used to train a multi-class Support Vector Machine (SVM) classifier. The therapeutic class of each drug has been extracted from the Anatomical Therapeutic Chemical classification (ATC). The ultimate ranking for

each medication was therefore obtained by selecting the most persistently predicted ATC classes.

The authors found multiple interesting repositioning possibilities for the selected drugs portfolio included in this study. They identified benzimidazole antihelmintics, that belong to a group of anti-parasitic drugs, which interfere with microtubule synthesis in the parasites, could be potent anti-neoplastic medications inducing mitotic arrest in tumor cells. Interestingly such antihelmintics as mebendazole and albendazole were previously reported to have microtubule activity in cancer cells. In cell culture, depending on the concentration, albendazole was shown to induce cancer cells arrest in the G0–G1 (at low doses) or the G2–M (at high doses) phases of the cell cycle. Inhibition of tumor growth *in vivo* was shown in various tumor types [223] and was tested in a phase I clinical trial in patients diagnosed with advanced solid tumors [224].

3.5.4. Diseasome networks

Many other computational approaches build disease networks linking specific symptoms with underlying molecular interactions [225]. For example, a "diseasome" network of disease-disease relationships connecting genetic origins, symptoms, markers and comorbidities was recently presented [226]. This approach aimed to merge interrelations between various diseases and define them *de novo* by their molecular mechanisms and not symptoms. Such data-based method clustered a disease phenotype containing protein interactions, molecular mechanisms, or gene-disease interaction and associations.

We are aware it is not an exhaustive list of all integrative approaches present in the literature. By creating this short list of five selected studies using novel integrated approaches based on machine learning algorithms we intended to signal the importance of these critical approaches. It becomes therefore crucial for biomedical research to elaborate the tools that will efficiently integrate multiple parameters from very large datasets in order to build a high-quality map of their complex network in populations as well as in individuals.

3.5.5. Drug-target-disease networks

Since the therapeutic and drug adverse effects depend strongly on the inhibition or activation of target protein (s), a method for predicting inhibitory and activatory effects of drug candidates has been recently proposed by Sawada *et al.* [227] through the integration of phenotypic, transcriptomic and genetic information. This fifth strategy contains comprehensive drug-target-disease networks where negative and positive targets are distinguished for drugs and diseases. The authors integrated chemically-induced and genetically-perturbed gene expression profiles in human cell lines, independent of chemical structures of compounds or proteins. Simultaneously, they used a learning algorithm using transcriptomic changes in global gene expression profiles registered after chemical treatments, together with knock-down and over-expression of proteins.

Using this learning method, the authors hypothesized that if a drug inhibits a certain protein, the following gene expression profile might be linked with the one after gene knock-down of this corresponding protein as in chemical genetics. Similarly, after the activation of a protein of interest by a drug, the gene expression after treatment (chemical treatment signature) with the compound might very well be correlated with that after over-expression of a protein as in gain of function approaches. Individual predictive models were therefore constructed for multiple target proteins, and the model's simultaneous learning was achieved by sharing protein similarities based on gene knock-down and over-expression profiles. Correlation coefficients for inhibitory interaction pairs were calculated from chemical treatment and gene knock-down signatures, whereas for activatory interaction pairs were calculated based on chemical treatment and gene over-expression signatures. As a result, the method predicted drug-target-disease association networks for over a thousand drugs and over 800 target proteins in 365 human diseases. Focusing on human retinoic acid receptor alpha (RAR α) as a target, they used a one-hybrid GAL4-reporter gene assay and a drug candidate predicted to inhibit RAR α, successfully validating the prediction in this assay.

3.5.6. Bayesian networks

Another type of algorithm used in very large-scale drug combination studies is a Bayesian network that is, in general, a representation of the joint probability distribution among multiple variables [228]. This method, however requires more experimental input as compare to the Differential Evolution used in the FSC.

Li *et al.* who used a similarity algorithm together with Bayesian network termed Probability Ensemble Approach (PEA) in order to model the possible drug combinations using the information on a drug molecular and pharmacological similarity [229]. In the next step of this large-scale search, the authors assessed the obtained predictions of drug combinations with adverse effects and clinical efficiency. Fifty five predicted drug pairs were tested in human non-small cell lung cancer cells, where 39 drug pairs were found synergistic or additive. Overall, this method deals with the activities of drugs and their clinical effects in the context of molecular network systems, with the latter also yielding information on how to further understand the molecular mechanism and pharmacological effects of a drug combination. It is important to note that most drugs are selected to the combinations based on their individual performance in clinical trials. Moreover, the drugs are being combined with the medications belonging to the same therapeutic class, e.g. hypertension drugs. This type of selection implicates the similar functions and narrows to possibility of finding synergism. The method described above was unbiased by the functional similarities of two drugs in a combination, as 43% of identified drug combinations contained drugs from different drug classes.

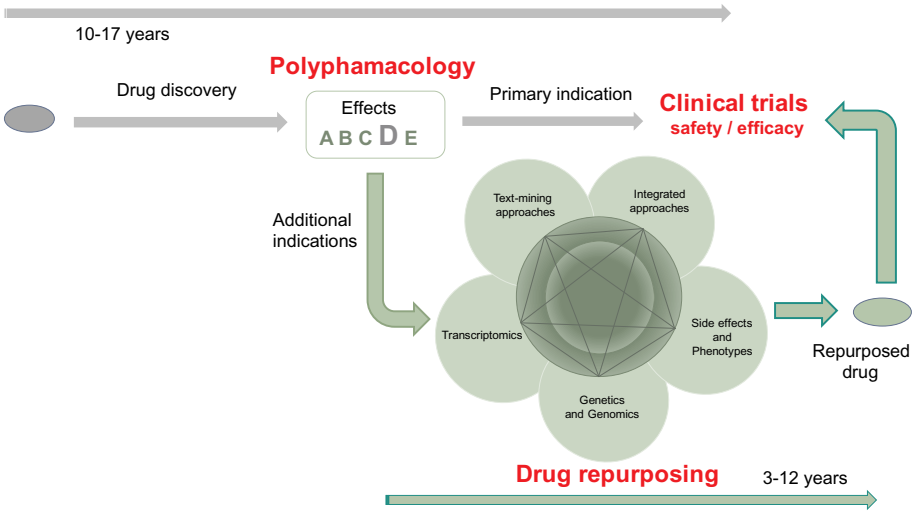
4. Conclusions and future directions

Drug repurposing in oncology promises to have a high impact on a significant number of patients through their ability to gain access to novel, fast-tracked personalized treatments. Innovative phenotypic and omic assays, patient-specific testing, novel screening methods with well-defined primary cancer cells, patient-derived grafts, and machine-learning algorithms to speed up discovery may quickly yield new drugs for multiple treatments based on the principle of polypharmacology and refined phenotyping, critically taking into consideration tumor cell heterogeneity. When repurposed therapies demonstrate improved efficacy, safety and cost over the standard treatment of care, not only patients but both public and private organizations will benefit. Scheme 2 presents the classical drug development pathway and drug repurposing, described in this review.

The efficacy in clinical trials, however, remains the major bottleneck for successful drug development. This, in our view, may be in part due to variable, often subpar assays commonly used such as 'cell death on a dish' and to the heterogeneity of primary tumors and metastases. Better and more informed phenotypic assays before clinical trials are required to increase the success rates.

Several promising approaches based on clinical symptoms, genomic and transcriptomic data, as well as databases (e.g. SIDER, PharmGKB) have been developed and new ones are quickly being introduced. Promptly developing computational approached merging different "-omics" and multi-network multi-layered data sets, will facilitate future repurposing of drugs for multiple oncological indications. As already demonstrated [230,231], repurposing drugs in combination with other agents could potentially improve clinical outcomes in cancer patients.

As a point of caution, we wish to underline that repurposing drugs from medicine to veterinarian indications can be highly beneficial, but also have negative effects on the environment depending on the scale of deployment: For example, massive veterinarian use of ivermectin on farm and range animals can have unwanted negative effects on the



Scheme 2. Classical drug development pathway and drug repurposing options.

ecosystem (e.g. [232,233]). Similarly, massive veterinarian use of diclofenac almost wiped out the Indian vulture population feeding on carcasses of treated animals, leading to the ban of this substance for veterinary use (e.g. [234,235]). Another concern is the massive buildup of medicinal residues (endocrine disruptors, antibiotics, nanoparticles) in the water and its effects on human beings and the ecosystem (e.g. [236,237]). Given the rising number of cancer patients, environmental and ecosystem safety must be added the list of top concerns for finding effective anti-cancer therapeutics that enhance human lives in the widest sense in an era of nearly 8 billion human beings. While finding effective treatments for so far incurable diseases remains a key goal, prevention, including a healthy diet, reduced stress [174] and exercise [178,179] from early childhood should be a top priority

Given the pace of research and the need for innovative treatments for metastatic colorectal and other cancers, we suspect that major breakthroughs will be achieved in the next decades. This will derive from the improvement and use of machine-learning algorithms and the efficient mining of ever-enlarging datasets to predict novel drug-disease pairs, coupled to rigorous omic and *in vivo* phenotypic testing. Representative and predictive phenotypes should be identified, taking into consideration not only the cancer cells themselves as selected genetic entities of a specific patient, but also their evolving epigenetic status and the influences of the surrounding systems such as the tumor stroma, the immune system, the microbiota and hormonal and neural systems. A major challenge is thus not only to speed up repurposing of known drugs but to develop personalized phenotypic validation schemes.

Taken together, such a computationally predictive-drug discovery-phenotypic validation preclinical pipeline should present a prioritized short list of candidates to validate in clinical trials, yielding novel treatments for very old diseases that include colorectal cancers.

Acknowledgments

PNS acknowledges the European Research Council (ERC-680209) and Fondation pour la Lutte Contre le Cancer et pour des Recherches Médico-Biologiques; ARA's lab was funded by grants from the FNS, the League Suisse Contre le Cancer, EU-ITN CAPPELLA AND HEALING, and a James McDonnell Brain Cancer Award. All three authors acknowledge funds from the Department of Public Instruction of the Republic and Canton of Geneva, Switzerland. We thank Carlotta Cecchini for providing the chemical structures.

Competing interests

The authors have declared no competing interests

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