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Opportunities and challenges in combination gene cancer therapy

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Abstract

Treatment for solid tumor malignancies, which constitute the majority of human cancers, is still dominated by surgery and radiotherapies. This is especially true for many localized solid tumors, which are often curable with these treatments. However, metastatic cancers are beyond the reach of these therapies, and many localized cancers that are initially treated with surgery and radiation will recur and metastasize. Thus, for over 60 years there has been a concerted effort to develop effective drug treatments for metastatic cancers. Combination therapies are an increasingly important part of the anti-cancer drug armamentarium. In the case of cytotoxic chemotherapy, multi-drug regimens rapidly became the norm, as the earliest single agents were relatively ineffective. In contrast to chemotherapy, where combination therapies were required in order to achieve treatment efficacy, for both hormonal and targeted therapies the impetus to move toward the use of combination therapies is to prevent or reverse the development of treatment resistance. In addition, emerging evidence suggests that combination therapy may also improve cancer treatment by neutralizing an emerging treatment side effect termed therapy-induced metastasis, which accompanies some effective single agent therapies. Finally, although gene therapy is still far from use in the clinic, we propose that combination therapies may enhance its effectiveness.

Graphical abstract

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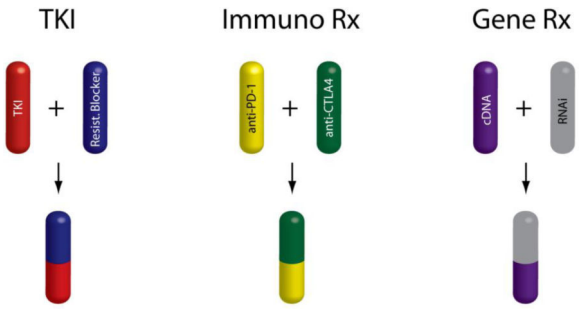

Keywords

Cancer; therapeutics; chemotherapy; hormonal therapy; targeted therapy; gene therapy; death receptors; c-FLIP

PURPOSE

The purpose of this set of reviews is to describe advances in the development and deployment of multi-modal therapies. Most of the reviews focus on exciting new technologies that can deliver two or more drugs to patients, including those with cancer. This review will complement those discussions of advanced drug delivery technologies by describing some of the biological, clinical and practical arguments that favor the use of combination cancer therapy and suggest that such combination therapies will be superior to the still widely-employed cancer treatment paradigm of serial application of single therapeutic modalities. While the accompanying reviews discuss many of the technical impediments and opportunities in the development of multi-modal drug delivery, here we will review the other challenges in effectively implementing combination cancer therapies. Specifically, we will identify drug therapy combinations that make good biological sense as well as when and where novel multimodal therapy delivery is required for effective combination treatment, rather than simply delivering two drugs simultaneously by established oral or intravenous routes. Therapeutic modalities for cancer can be divided into seven types: surgery, radiotherapy, chemotherapy, hormone therapy, the recently developed class of targeted therapy, the emerging category of cellular (immune) therapy, and finally the still more or less exclusively experimental category of gene therapy. There are other possible categories (such as drug-based immunotherapy) and there is definitely overlap among these categories, but this classification facilitates our discussion of the biological basis for advanced multi-modal delivery technology.

Three of these seven treatment modalities – surgery, radiotherapy and cellular therapy – are not relevant in the context of the advanced delivery technologies discussed in this issue, and therefore this review will focus on the remaining four categories of drug-based therapies. However, it is useful to mention that surgery, and to a somewhat lesser extent, radiotherapy have historically been frequently used in combination with drug therapies in the treatment of cancer. Specifically, drug therapy given soon after excisional surgery of the main tumor mass is referred to as adjuvant cancer therapy, which is widely employed to eliminate residual disease, typically micro-metastatic cancer. Cellular immune therapy might serve a similar

adjuvant role although this application is still in its infancy [1]. Similarly, neo-adjuvant drug therapy is applied prior to surgical excision, often to reduce the bulk primary tumor mass prior to surgery. Radiation is one of the most common types of neo-adjuvant therapy. Finally, radiation has been used in combination with drug therapies to sensitize tumors to the effects of local radiation, allowing lower regional radiation dosing and thereby preserving normal tissue surrounding the site of radiation [2].

AN ABBREVIATED HISTORY OF COMBINATION CANCER DRUG THERAPIES

Chemotherapy

As detailed in Mukherjee's comprehensive and illuminating history of cancer [3], beginning as far back as medieval times, treatment focused on improving the efficacy of surgery and to a lesser extent (and much later) on radiotherapy, with the occasional application of cell therapy to stimulate anti-tumor immunity (e.g., Coley's toxins [4,5]). Following WWII a number of single agent chemotherapeutic drugs were developed. These were either metabolic or DNA replication poisons sometimes referred to as 'cytotoxic' chemotherapies. These early drugs demonstrated only limited value in human clinical trials as single agents, but by the 1960's successful treatments were devised using combinations of these drugs [6]. A key scientific breakthrough was the realization that efficacy (mainly assessed in rapidly proliferating cancers) was dependent on highly efficient tumor cell killing, which was best achieved employing multiple agents simultaneously (or in rapid cycles) [6]. It was also critical that the different component agents constituting the combination therapy 'cocktails' act by distinctly different cytotoxic mechanisms [7]. Thus, similar to the use of multiple anti-microbial agents in stubborn infectious diseases such as TB (and later HIV), multi-modal chemotherapy for cancer became widely employed in many human cancers [7].

Hormone therapy

In contrast to chemotherapy, many of hormonal and targeted anti-cancer drugs have been successfully employed as single agent therapies. However, it has become increasingly clear that while these single agent therapies extend patient survival, resistance eventually develops. Therefore, these classes of therapy are increasingly being combined with other drug therapies in order to prevent the development of resistance. Hormonal therapies typically interfere with the binding of the steroid ligands to the family of nuclear hormone receptors, most notably the androgen and estrogen receptors, in prostate and breast cancers, respectively. As an example, we describe the evolution of hormonal therapeutic strategy in prostate cancer, the leading cause of cancer in US men [8]. Huggins and Hodges demonstrated in 1939 that androgen deprivation therapy (ADT) by castration was effective in the treatment of metastatic prostate cancer [9]. Subsequently, 'chemical castration', employing gonadotropin releasing hormone agonists such as leuprorelin that lead to reduced androgen levels via feedback inhibition, has largely replaced castration surgery. While ADT is initially effective in the treatment of metastatic prostate cancer, there is near universal recurrence as a form of prostate cancer referred to as castration resistant prostate cancer (CRPC), which is usually fatal within two years [10–13]. Since 2004, multiple agents have

been approved for CRPC, including docetaxel chemotherapy [14], the anti-androgen enzalutamide [15–17] and the androgen biosynthesis inhibitor abiraterone [18,19]. These have been employed as single agents, and are often used serially, but all ultimately induce resistance [20–23], re-activating tumor growth. Within the past year, the combination of chemotherapy (docetaxel) plus ADT has significantly increased overall survival [24,25], likely marking the beginning of routine combination drug treatments for CRPC. Moreover, an ongoing trial will determine the value of using a combination of the anti-androgen enzalutamide and the androgen biosynthesis inhibitor abiraterone in the same patient population (https://clinicaltrials.gov/ct2/show/NCT01949337).

While resistance is a frequent reason for the failure of single agent hormonal therapy, it may not be the only cause. Single agent hormonal therapies such as leuprorelin and enzalutamide appear to induce resistance via multiple mechanisms, including i) mutation in the androgen receptor (AR) ligand binding domain, creating variant AR proteins that can recognize an anti-androgen (antagonist) as an agonist [22,23,26]; ii) epigenetic mechanisms that increase the relative expression of AR splice variants, such as those encoding ligand-independent versions [27] and iii) the up-regulation of WNT pathway signaling genes which are known to drive proliferation in other cancers [28]. However, these same hormonal treatments of advanced prostate cancers may result in the development of a type of treatment failure that has been described as ‘treatment-induced metastasis’ (TIM) by Ebos [29]. For example, castration and enzalutamide increase metastasis in both mouse and cell culture models of human prostate cancer, via the induction of the chemoattractant protein CCL2, which promotes migration of tumor cells and infiltration tumor-associated macrophages [30–34]. The observation of TIM in model systems is supported by some initial patient studies [32,35,36], indicating that combination therapies that include either anti-CCL2 monoclonal antibodies [37–40] or anti-CCR2 inhibitor [41] represent an additional opportunity to increase the efficacy of leuprorelin or enzalutamide in CRPC. Radiation therapy of prostate cancers increases expression of the AR, and reduces patient survival, suggesting that another single agent treatment for prostate cancer produces TIM and might effect might be reversed by additional combination therapies, such as the use of enzalutamide immediately following radiotherapy [36], and might also account for improved survival for patients receiving extended ADT following radiotherapy.

Targeted therapy

This category [42] is predicated on the concept of oncogene addiction, first postulated by Weinstein [43]. He reasoned that inhibition of a single mutant driver oncogene is sufficient to induce cancer cell death in those cancers in which sustained proliferation is ‘addicted’ to constitutive activation of the corresponding signaling pathway. The grand-daddy of targeted therapy is imatinib (Gleevec), a tyrosine kinase inhibitor (TKI) that has been remarkable effective in controlling the growth of chronic myelogenous leukemia (CML) caused by the bcr-abl fusion oncogene produced by the so-called Philadelphia chromosome [44]. Examples of successful similar kinase targeted cancer therapies in the more prevalent ‘solid’ human cancers include gefitinib inhibition of mutant epidermal growth factor receptor (EGFR) in lung adenocarcinomas [45] and vemurafenib inhibition of V600E mutant BRAF genes in malignant melanoma [46]. According to the oncogene addiction paradigm, single

agent therapies are predicted to be sufficient, and indeed have demonstrated remarkable tumor regression as well as enhanced overall survival. Moreover, the advent of targeted therapy has required the coordinate application of advanced molecular diagnostic tests on individual tumor samples, often harnessing next-generation sequencing technology. Thus was borne the era of personalized or precision medicine.

However, the dramatic responses to targeted therapy are limited by pre-existing or acquired therapy resistance [47], with the former a likely consequence of selection of minor variants among a heterogeneous population of tumor cells [7]. Perhaps the best understood resistance mechanisms are secondary missense mutations in driver kinase oncogenes. The T315I mutation in bcr-abl, reduces imatinib access to the active site [48]. A similar ‘gateway’ mutation (T790M) in the EGFR accounts for many cases of clinical resistance to erlotinib and gefitinib, which target the TKI-sensitizing mutant forms of EGFR found in primary lung cancers [49–51]. A combination therapy, employing a kinase inhibitor and a monoclonal antibody drug, both directed at the EGFR, showed some effectiveness in patient tumors with the T790M EGFR mutation [52], but this has been displaced by a new single agent approach, employing a ‘third generation’ irreversible kinase inhibitor (AZD9291) [53]. In melanoma, at least three mechanisms cause resistance to the V600E BRAF inhibitors, two of which (PDGFB and MEK1) are druggable at this time [54,55]. In this case, initial success in treating resistant tumors that activate MEK1 signaling has been achieved using a combination of a BRAF inhibitor and a MEK1 inhibitor [56]. Finally, in the case of bcr-abl, second and third generation TKIs have not been entirely successful in treating resistant CML [57] but a combination of imatinib and a novel therapy directed against Stat3 has shown some success in pre-clinical studies [58]. Thus, combination therapies represent a likely approach to some, if not most, resistant cancers that occur following targeted therapy. Success seems to correlate with drugs designed on the basis of a detailed understanding of the underlying molecular mechanism of resistance, rather than a ‘mix-and-match’ approach with available drugs.

Checkpoint blockade therapy

It is also worth reviewing some of the most recent advances in immune-modulating drug therapy, which is distinct from cell-based immune therapy. This group of drugs may be considered an additional separate category of therapy or, alternatively, a targeted therapy (although the ‘target’ is typically on the surface of infiltrating T-cells in the tumor micro-environment). These drugs have been particularly effective in metastatic melanoma, but are being tested in a wide range of human cancers. The most promising of these are the checkpoint inhibitors, which target two cell surface molecules, CTLA-4 and PD-1 that negatively regulate T-cell activation [59]. The drugs, monoclonal antibodies directed against the respective cell surface molecules, have shown success in a number of cancers as single agent therapies. A striking feature of the early clinical trials of checkpoint inhibitors is the durability (length) of the responses elicited, which are generally significantly longer than those of the oncogene-targeted therapies described above [60]. However, the drugs are clearly not effective for every tumor in a given histological subset and therefore, as with other targeted therapies, there is a pressing need to identify biomarkers to identify those individual tumors that are likely to respond. Unfortunately, there has been limited success in

patient stratification by biomarkers thus far [60]. The first sets of clinical trails and FDA approvals have focused on single agent inhibitors (which have generally been quite successful). A phase I (safety) trial combining the BRAF inhibitor vemurafenib and the anti-CTLA4 inhibitor ipilimumab was halted due to toxicity [61]. In contrast, a combination of anti-CTLA4 and anti-PD-1 agents demonstrated an apparent synergistic effect in response durability in a phase III efficacy trial as a combination therapy for unresectable metastatic melanoma [62]. The mechanism of synergy remains unknown, and there are significant concerns about safety (systemic toxicity) when combining the two checkpoint inhibitors [62]. Nonetheless, this may signal a trend towards combination therapies among the wave of similar checkpoint inhibitor drugs under development, as these drugs are applied to a wider spectrum of histological tumor types.

CHALLENGES TO EMPLOYING COMBINATION THERAPY

Industry business models are a potential obstacle to implementing combination therapy for cancer. Specifically, it has been reported that many pharmaceutical companies are reluctant, for a variety of sound business reasons, to collaborate in the trials and co-marketing of combination drug therapies, when different companies own the individual agents [61]. For instance, two large pharmaceutical firms have independently developed both a BRAF inhibitor and a MEK1 inhibitor for use in a combination therapy aimed at resistant metastatic melanoma [61]. Another example is the acquisition of Aragon Pharmaceuticals and its anti-androgen ARN- 509 by Johnson & Johnson, apparently to provide an alternative to enzalutamide (owned by Astellus/Medivation) in the development of a combination therapy with the androgen synthesis inhibitor abiraterone (http://www.investor.jnj.com/releasedetail.cfm?releaseid=771753). The flip-side of Pharma concerns about controlling both agents in a combination therapy regimen, are consumer and health care insurance industry concerns about the escalating costs of oncology drugs, which are of course compounded by a combination therapy using two (or more) high-priced anti-cancer drugs [63]. If healthcare providers are prevented by insurance coverage limitations from using high-priced combination therapies for cancer, drug developers may focus on pairing high-priced targeted therapy drugs with more affordable cytotoxic chemotherapies. So far the science seems to argue against most such combinations.

These potential financial limitations cannot, of course, be solved by improvements in drug delivery. In contrast, in the case of chemotherapy, multi-agent treatment regimens are well-established [7], and the major challenge to maximizing efficacy is minimizing toxicity. Clinical studies have optimized dose and frequency, and many combinations employ cytotoxic drugs that act via different mechanisms to increase cancer cell killing, and reduce toxicity (and the development of therapy resistance) [7]. Nanoparticle co-delivery might be an approach to reducing systemic side effects, since the drugs could be contained within the particles until reaching the intended target. Unfortunately, initial attempts at co-delivery of two hydrophobic chemotherapeutic drugs have run into unexpected problems such as physic-chemical interactions between the drugs [64]. Combinations that use one hydrophilic drug (erlotinib) and a hydrophobic chemotherapy drug (doxorubicin) have been delivered in a time-staggered manner via nanoparticles [65]. Overall, there is a consensus that cytotoxic chemotherapy has reached, or is nearing, its effective limits and most cancer drug

development is focused on therapy that exploits our understanding of differences between the biology of normal and cancer cells and the tumor microenvironment. However, any multi-modal delivery system that can more specifically target cytotoxic chemotherapy – presumably by delivering therapy to tumor cells via a molecular tag – should be able to enhance chemotherapy effectiveness by reducing exposure to normal, non-tumor tissues.

A PROPOSAL FOR DUAL GENE PRO-APOPTOTIC THERAPY

Gene therapy is the final category of drugs to be reviewed here, in recognition of the fact that that no cancer gene therapy drug has been approved in the US, although two have been approved in China [66]. Tumorigenesis is a complex process involving multiple tumor specific and host specific molecular processes [67] but at the level of the tumor cell the process of carcinogenesis is driven by two classes of genetic alterations: oncogene activation and the loss of tumor suppressor gene expression due either to deletion, inactivating mutations or epigenetic silencing of both alleles in the cancer cells. Extrapolating from the success of therapies targeting kinase driver oncogenes, we might predict that restoring expression of a single tumor suppressor gene should have the same dramatic effect. Thus, a major candidate gene therapy has been the tumor suppressor p53, which is lost in many human tumors [68]. Despite a substantial effort, p53 gene delivery to tumor cells has not been successful in the clinic [66]. Phase I (safety) testing was successful, but not efficacy testing trials. While a similar therapy is one of the two gene therapies approved in China, its efficacy is not yet characterized [68]. Hurdles to gene therapy in the US are expected to be significant given the safety issues (i.e., inadvertent induction of leukemia) that plagued the X-SCID gene therapy trials over a decade ago [69,70]. Since these were likely due to insertional mutagenesis, precise genome editing technologies (TALEN, CRISPR) offer the most hope for future FDA approval [71].

One possible reason for the failure of p53 gene therapy is inefficient restoration due to poor delivery or a failure to be adequately expressed in the tumor cells. This assumption has undermined confidence in the gene therapy approach and has lead a number of investigators to suggest indirect or alterative approaches to restoring tumor suppressor gene function, that do not involve direct gene replacement [72,73]. Only about 1 in 20 drugs entering phase I human clinical trials for cancer are eventually approved [74]. The major impediment appears to be a lack of efficacy in humans, rather than safety issues. Problems with pre-clinical trial reproducibility may be a cause of the low rate of drug development success [75,76], but it could also be that murine models, even those genetically engineered to resemble human cancers, are not adequate models, and therefore restoration of a single tumor suppressor gene is simply not sufficiently potent in the more complex genetic landscape of human tumors. This suggests that targeting multiple genes might improve the chances of success for gene therapy. As we harness the new gene delivery technologies described in this issue, it is important to identify biologically rational targets, since it will be impossible to systematically test all potential two-gene combinations [7]. Moreover, a key advantage of gene therapy is the ability to target otherwise undruggable targets, providing the opportunity to select biologically relevant genes without restriction.

Castration resistant prostate cancer is driven by a variety of mechanisms that reactivate AR signaling [77]. Newer therapies for CRPC (e.g., enzalutamide and abiraterone) target these reactivation mechanisms and prolong survival. However, treated tumors ultimately develop resistance to these new drugs [21,22], restoring the tumor to an androgen receptor-dependent state, and re-activating tumor growth. Using the insight gained from mechanistic studies of androgen withdrawal induced prostate apoptosis, we outline a possible pro-apoptotic therapy that acts downstream and independent of AR signaling, thereby inducing cell death in metastatic prostate cancer even when AR signaling has been re-activated. Specifically, we propose that apoptosis (pro-apoptotic therapy) can be induced in prostate cancer cells by simultaneously over-expressing pro-apoptotic TNF and silencing anti-apoptotic c-FLIP (CFLAR).

Death receptors are transmembrane proteins, which bind a family of ligands that can induce cell death via activation of caspase-8 [78]. TNF is the prototype death receptor ligand, trimerizing TNFR1 and subsequently activating caspase-8 to induce apoptosis (Fig. 1) [79]. TNF can also stimulate cell survival in the context of inflammation (Fig. 1, center pathway). c-FLIP, a dominant negative inhibitory homologue of caspase-8, is a key regulator of TNF signaling, effectively blocking caspase-8 activation and switching TNF signaling towards survival. c-FLIP is transcriptionally down-regulated by androgen withdrawal, implicating this key inhibitor of death signaling in androgen withdrawal induced prostate apoptosis such as occurs in ADT [80–86]. Furthermore, CRPCs have elevated levels of c-FLIP [86–88]. Since lowering c-FLIP sensitizes cells to apoptotic stimuli it is expected that therapeutic c-FLIP silencing will effectively contribute to pro-apoptotic therapy for metastatic prostate cancer. Indeed, Korneluk and colleagues have demonstrated that c-FLIP silencing in a wide variety of cancer cell lines sensitizes cells to TNF (and TRAIL, another death receptor ligand) induced apoptosis [89,90], strongly suggesting that c-FLIP silencing must be combined with death receptor ligands to efficiently induce cancer cell death [91]. In a separate study [92] we have shown that TNF is required for castration-induced prostate regression and apoptosis, while other death receptor ligands (TRAIL, FasL) are not. Moreover, following castration TNF is up-regulated in prostatic stromal cells and acts in a paracrine manner to induce apoptosis of the epithelial cells. We can induce apoptosis of prostate epithelial cells *in vitro* by exogenously delivering TNF and silencing FLIP, bypassing the androgen regulation of the promoters of these two key genes. This suggests that the pro-apoptotic therapy can be achieved by sensitizing tumor cells, which have acquired high levels of c-FLIP expression, via c-FLIP silencing. Moreover, simultaneous expression of TNF will act to trigger the caspase activation via the TNFR1 receptor (Fig. 1).

Thus, our studies on the molecular mechanism of androgen withdrawal induced apoptosis have identified an androgen activated gene (c-FLIP) as well as an androgen repressed gene (TNF), each of which encode a key mediator of prostate apoptosis. The effect of androgen withdrawal is to up-regulate TNF and down-regulate FLIP; these events synergize to induce apoptosis (Fig. 1). We hypothesize that delivery modalities that can exogenously over-express TNF and silence c-FLIP, will induce apoptosis while circumventing the androgen axis dependency of these genes. However, there are significant delivery challenges to overcome. A number of drugs have been found to down-regulate c-FLIP but these are likely to have multiple effects on both cancer and normal cells and may prove to be toxic [93].

Therefore, a means of specifically silencing c-FLIP (e.g., RNAi) is probably required to make this therapy safe and effective. Systemic administration of TNF is toxic [94], although this toxicity can be significantly reduced by intra-lesional administration [95]. This suggests that a means of restricting TNF expression to prostate cancer cells (e.g., using a prostate-specific promoter) will also be required (although it might be possible to use nano-carriers to deliver TNF protein in a targeted manner [96]). If prostate cancer cell-targeted, dual gene delivery, can be achieved then we hypothesize that the proposed therapy will be effective for tumors that are resistant to ADT and/or recently approved anti-androgenic drugs.

CONCLUSIONS

Combination therapies greatly improved the effectiveness of cytotoxic chemotherapy, but it has only recently been implemented for hormonal, targeted and immune therapies, mainly as a means to evade the inevitable development of resistance. We speculate that combination gene therapy, if it can be delivered efficiently, may make gene therapy a more viable option for some cancer treatments.

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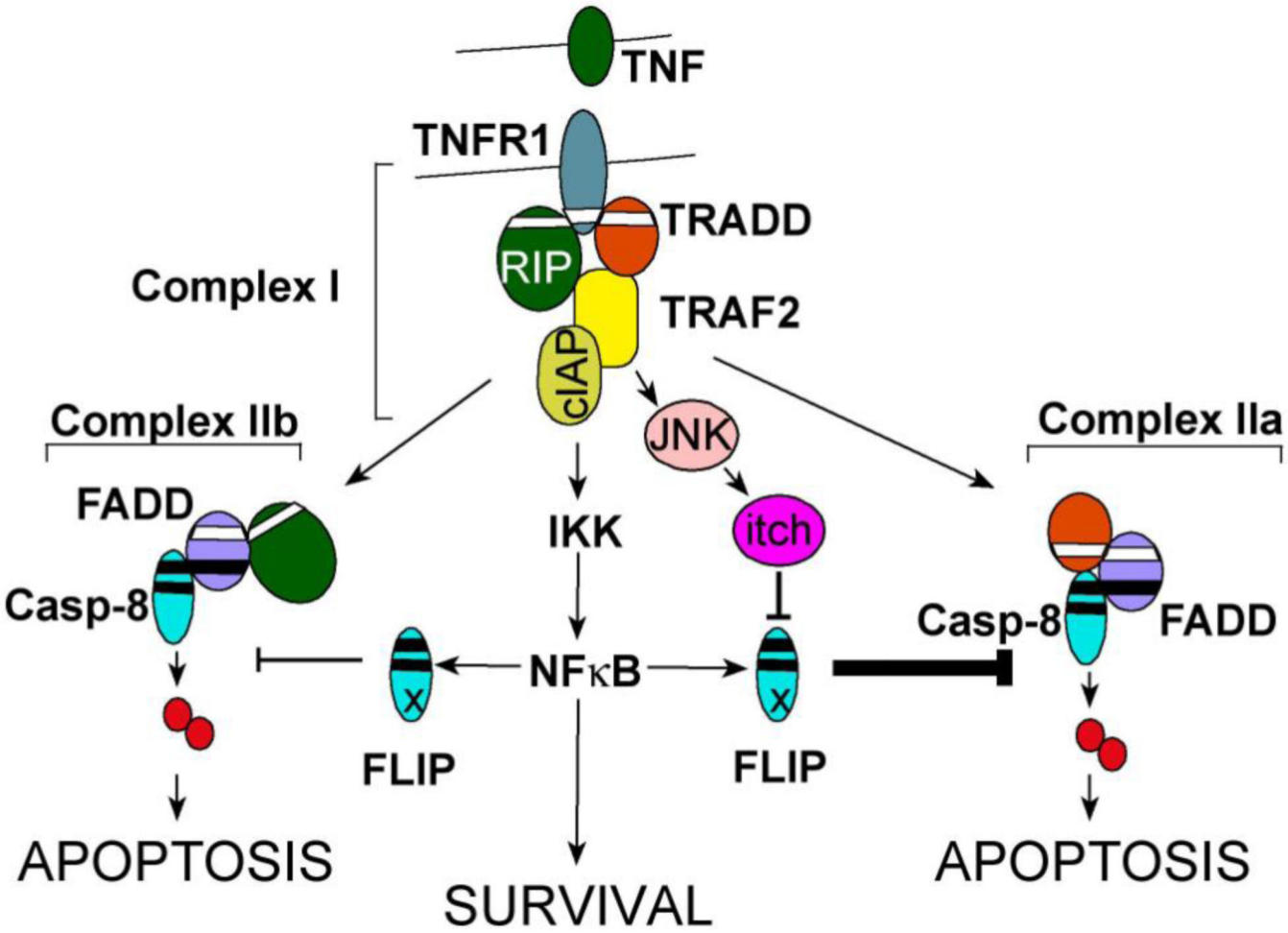

Figure 1. TNF signaling network

TNF can signal either survival or apoptosis via its receptor, TNFR1. NFκB and the caspase-8 inhibitor c-FLIP (FLIP) are key intracellular regulators that control the switch between apoptosis and survival signaling.