Antisense Approaches Enter the Clinic

Fadlo R. Khuri and Jonathan M. Kurie
Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

The last decade of the twentieth century saw an explosion in the application of molecular biology toward developing pathways for rational drug design (1). Because Krontiris and Cooper demonstrated that h-ras was capable of transforming malignant cells (2), and because the ras pathway is among the most studied in human and mammalian cancer systems, it was natural that several of these approaches should target the ras oncogene (3–5). Approaches to targeting the ras pathways have been abundant. Perhaps the most extensively studied include the development of farnesyl transferase inhibitors, developed initially as a potential anti-ras therapy. As information about the complexity of the signaling cascade continued to emerge, other important targets were selected for preclinical development.

The number of approaches targeting the signaling cascade that are in clinical trials is impressive. Small molecule development has accelerated at an astonishing pace. Currently, several different agents inhibiting specific tyrosine kinases and several pharmacologically different farnesyl transferase inhibitors are in clinical trials (6–8). In this setting, antisense approaches to both raf-1 (9, 10) and protein kinases C and A (11, 12) have been introduced into the clinic during the last few years, leading to the reporting of four different trials. Some of these trials have even reported durable clinical responses. However, what remains to be seen is not so much what the initial findings of these trials will show but in what directions they will lead us. As the debate continues to swell regarding the role of these new molecules as stand-alone single agents or in combination with chemotherapy, their ultimate role has yet to be determined.

ras/raf targeting approaches are highly attractive to the clinical investigator. To begin with, the ras oncogene is dysregulated or frankly mutated more frequently than any other oncogene studied in human cancer (13, 14). Furthermore, in several tumors, including non-small cell lung cancer, it has an important prognostic role (15). In addition to this, in certain tumors such as pancreatic cancer, where standard treatments are strikingly ineffective, these approaches are particularly attractive because 95% of these tumors have ras mutations (13). This preponderance leads one to believe that this is a critical oncogenic change in carcinogenesis and potentially in overall outcome.

Antisense Therapy: Rationale and Limitations

The critical parameters in drug design have long been the identification of an appropriate target in a disease process and the development of a targeted agent with specific recognition and affinity to the above-mentioned target. Furthermore, the target of the proposed therapy must be one critical for disease progression, the inhibition of which can lead to reversal or stabilization of that disease. Unfortunately, the vast majority of drugs discovered or designed to date recognize their target by mechanisms that are often poorly defined and only moderately specific at best. Antisense oligonucleotides can inhibit the expression of specific genes by binding to mRNA transcripts, with extensive in vivo and in vitro data indicating that this is highly achievable (9–12).

The specific advantages of this approach over conventional drug design include targeting the mRNA of the specific disease target gene with a unique sequence tailored for that gene sequence and the interaction of the antisense oligodeoxynucleotide with the target gene by Watson-Crick base pairing, thereby providing specificity and affinity. However, despite this potential advantage of specificity, delivering the antisense molecule to the appropriate target is the far greater challenge. Evidence persists that the phosphodiester backbone of native oligodeoxynucleotides is highly susceptible to rapid degradation by nuclease in human serum (9). Therefore, as these and other authors have noted, systemic administration of antisense oligodeoxynucleotides for therapy requires critical chemical modifications conferring some degree of resistance to nuclease activity to enhance the stability of in vivo systemic administration and achieve meaningful binding. The substitution of a sulfur for an oxygen in the phosphodiester linkage between nucleotides created a phosphorothioate, a structure that was significantly more resistant to rapid oxidative degradation. raf was selected as a target in this trial because of the frequency of raf and ras mutations in human tumors (13, 14), emphasizing their importance in the process of oncogenic transformation. Preclinical evidence suggests that these c-raf antisense oligodeoxynucleotides have activity in both human leukemia and solid tumors in both cell lines and xenografts.

Antisense Clinical Trials

This provocative study by Cunningham et al. (9) represents an important stride. ISIS 5132, an antisense oligonucleotide against c-raf, was well tolerated at doses up to and including 4.0 mg/kg/day administered by a 21-day continuous infusion. These doses of 2.0–4.0 mg/kg are apparently comparable to doses in mice at which activity was observed in human tumor xenografts. Notably, in this trial, one patient with refractory ovarian cancer had a dramatic reduction in her CA-125 level, and two other patients had prolonged disease stabilization for 9 and 10 months, respectively. There was a 97% decrease in CA-125 level. None
of the feared disruption of the coagulation cascade that had been suggested by preclinical data was clinically apparent in this study. Stevenson et al. (10) had previously reported a clinical/pharmacokinetic trial of this agent, with doses ranging from 0.5–6.0 mg/kg. The only clinical toxicity seen there was fever and fatigue, neither of which were dose limiting, and a clinically defined maximum tolerated dose was not achieved. In that trial, two patients experienced prolonged disease stabilization for more than 7 months. In both of these cases, this was associated with persistent reduction in c-raf-1 expression in peripheral blood mononuclear cells. The fact that responses in the Stevenson trial (10) were associated with significant decreases in c-raf-1 expression in peripheral blood mononuclear cells indicated a striking degree of specificity for this therapy.

Two additional studies are worth mentioning. Trials assessing protein kinase C-α and protein kinase A have also been launched. A trial by Yuen et al. (11) evaluating an antisense oligonucleotide to protein kinase C-α (ISIS 3521/CGP64128A) also achieved doses that were equivalent to pharmacologically active doses against human xenografts in mice. Also in this study, tumor responses that lasted up to 11 months were seen in three of four patients with ovarian cancer. The side effects were modest and consisted of thrombocytopenia and fatigue.

**Receptor Tyrosine Kinase Pathways as Modulators of Chemotherapeutic Response**

These clinical trials have demonstrated the potential of signal transduction inhibitors as single agents in cancer treatment. In addition, preclinical studies have demonstrated the potential of combining conventional cytotoxic chemotherapeutic agents with inhibitors of receptor tyrosine kinase signaling pathways. For example, recombinant humanized anti-HER2 antibody enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu-overexpressing human breast cancer xenografts (16, 17). Similarly, anti-EGF2 monoclonal antibodies augment the antitumor effects of cis-diamminechloroplatinum and doxorubicin on squamous cell carcinoma and breast cancer xenografts, respectively (18, 19). These approaches have been instituted into clinical trials that are currently in progress.

The mechanism by which blockade of the receptor tyrosine kinase pathway enhances the antitumor effect of cytotoxic agents has been the subject of intense investigation. Blockade of EGF receptor activation with the C225 inhibitory monoclonal antibody is associated with a rise in the levels of the p27 CDK inhibitor, which inactivates CDK-2 and produces cell cycle arrest in G1 (20). Furthermore, there is evidence that activation of receptor tyrosine kinase pathways induces resistance to chemotherapeutic treatment. Paclitaxel induces apoptosis of breast cancer cells by activating p34Cdc2 kinase, leading to cell cycle arrest at the G2-M phase and subsequent apoptosis (21). Overexpression of the receptor tyrosine kinase p185ErbB2 confers resistance to paclitaxel in breast cancer cells by increasing levels of the p21 CDK inhibitor, which associates with p34Cdc2 and inhibits paclitaxel-induced p34Cdc2 activation, delaying entrance to G2-M phase (21). Blockade of the increase in p21 levels induced by p185ErbB2 overcomes resistance to paclitaxel in
breast cancer cells (21). Thus, efforts to block receptor tyrosine kinase signaling at the cytoplasmic membrane receptor or at points downstream, including Raf or other receptor-activated kinases, are potentially effective means of enhancing the antitumor effects of cytotoxic chemotherapy.

Clinical trials evaluating this combination of the C225 monoclonal antibody with both chemotherapy and radiation are ongoing. In a Phase I study, Mendelsohn et al. (22) combined C225 with cisplatin in patients with recurrent head and neck squamous cell cancer. In addition to the evaluation of safety and tumor responses, the pharmacodynamics of EGF receptor saturation and persistent tumor EGF receptor inhibition were evaluated. Of the nine patients evaluable for response, six had major responses, including two complete responders (22). Three of the six responses were in patients who had previously failed cisplatin-based chemotherapy.

Ezekiel et al. (23) evaluated C225 in combination with either once or twice daily irradiation for locally advanced head and neck malignancies. A standard dose escalation procedure was used with each increment of C225 being given with 70 Gy of radiation therapy, except for the final two patients, who received 76.8 Gy. Of the 15 evaluable patients, 14 (93%) achieved complete responses based on physical and endoscopic examination. These encouraging results are in the process of being verified in a Phase III randomized trial.

The Complexity of Raf as a Target in Antitumor Therapy

Whereas these findings demonstrate tremendous potential for targeting receptor tyrosine kinase pathways in cancer therapeutics, recent studies demonstrate that these pathways are a complex network with apparently contradictory functions, which increases the difficulty of developing effective therapeuthic strategies. This complexity is partly the consequence of the signaling components themselves. For example, Raf-1 does not act alone; it is one component of a multiprotein complex (Fig. 1; Refs. 24 and 25.) Several Raf-associated proteins enhance Raf activation, including KSR and connector enhancer of KSR, whereas Raf kinase inhibitor protein blocks Raf-induced pathway activation. 14-3-3 is a Raf-associated protein that is important in both the activation and inhibition of Raf activity. The interactions of Raf-1 with proteins in this complex regulate the ability of Raf-1 to function as a serine-threonine kinase, which is central to its oncogenic activity.

The efficacy of targeting Raf or other components of the receptor tyrosine kinase pathways in cancer treatment will ultimately depend, in part, on the downstream pathways that they block. Raf-1 has multiple substrates that impact directly or indirectly on cell growth, including MEK-1, the retinoblastoma protein, and undoubtedly others that have not yet been discovered (26, 27). The mechanisms that regulate the specificity of Raf-1 substrate binding, including the role of Raf-associated proteins, have not yet been elucidated. Insight into these questions will be required to develop more potent and selective inhibitors of pathways that drive cancer cell growth.

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