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CONTUSUGENE LADENOVEC

Prop INN; USAN

Apoptosis Inducer
Cancer Gene Therapy

Ad5CMV-p53
AdCMV-p53
Adp53
INGN-201
RPR-201
RPR/INGN-201
Advexin®

Recombinant adenovirus that carries an expression cassette containing cytomegalovirus E1 promoter, human wild-type *TP53* cDNA and SV40 early polyadenylation signal

A modified human adenovirus 5 vector containing a cytomegalovirus promoter and a human *TP53* gene spliced into the DNA genome in place of the adenovirus E1A and E1B genes

(Recombinant) replication-restricted adenovirus (type 5) vector, E1-deleted, partial E3 deletion, containing/expressing a wild-type *TP53* gene driven by a cytomegalovirus promoter

CAS: 600735-73-7

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ABSTRACT

Contusugene ladenovec (INGN-201, Ad5CMV-p53, Advexin®) is a replication-impaired adenoviral vector that encodes a wild-type TP53 gene driven by a cytomegalovirus promoter. Mutations in the TP53 gene are the most frequent genetic alterations in human tumors, occurring in almost 50% of all cancers. The p53 protein is essential in maintaining genetic integrity after DNA damage, and alterations in the p53 pathway greatly increase the probability of tumor formation. Contusugene ladenovec has demonstrated clinical activity, both alone and in combination with other chemotherapy or radiation therapy, in several types of tumors, including squamous cell head and neck cancer (SCHNN), non-small cell lung cancer (NSCLC), breast cancer and glioblastoma. In addition, interesting data now support the use of contusugene ladenovec in two new exploratory applications: cancer immunotherapy, to provide circulating cancer surveillance in patients with an intact immune system, and cancer prevention, to prevent recurrence and/or metastasis in patients with premalignant oral lesions.

BACKGROUND

The *TP53* gene is one of the most intensively studied tumor suppressor genes (1). It is located on human chromosome 17p13.1 and encodes a 393-amino-acid nuclear phosphoprotein (2). The protein product of the *TP53* gene plays a critical role in cell cycle regulation and the control of apoptosis (3, 4). In addition, p53 can induce cell differentiation and senescence under certain circumstances.

The p53 protein functions as a tetrameric transcription factor by binding to specific DNA sequences and transactivating or repressing a large group of target genes (5-8). The transcriptional target genes of p53 have been extensively explored. Genes transcriptionally activated by *TP53* include: *BAX*, a positive regulator of apoptosis, *MDM2*, a negative regulator of *TP53* function, thrombospondin-1 (*THBS1*), an inhibitor of angiogenesis, and *GADD45*, which plays a role in DNA repair. A key function of the p53 protein is to control the progression of cells from the G_1 to the S phase. The p53 protein transcriptionally activates p21, which inhibits cyclin-dependent kinases (CDKs) (9, 10). Inhibition of CDK activity appears to block the release of the transcription factor E2F, causing failure of activation of transcription genes required for S-phase entry.

Altered protein function (suggested by elevated expression) and/or mutation of the *TP53* gene is associated with poor prognosis in patients with a variety of malignancies (11-13). The presence of a *TP53* mutation may also identify patients more likely to be resistant to chemo- or radiotherapy (14).

Recent studies have reported the introduction of a wild-type *TP53* gene into human tumor cells with a mutant *TP53* genotype using a variety of delivery methods, including retroviral vectors, lipid complexes and adenoviral vectors (15). Initial trials in humans using retroviral *TP53* gene transfer via intratumoral injection showed no toxicity and demonstrated evidence of antitumor activity in three of

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nine patients with non-small cell lung cancer (NSCLC) (16). However, low transduction efficiency associated with the retroviral vector was a major limiting factor.

Adenoviral vectors have a number of positive and negative features (17). Positive attributes are: 1) the transduction of a wide variety of cellular phenotypes, including not only epithelial and carcinoma cells, but also hematopoietic cells; 2) a high frequency of transduction and high levels of transgene expression; 3) the ability to accommodate large segments of DNA; and 4) a low pathogenicity profile for humans. Another important characteristic of adenoviral vectors is their lack of integration into the human genome. The adenoviral genome remains in the nucleus of the target cells as a nonreplicating extrachromosomal entity, avoiding any potential for mutagenic effects caused by random integration into the host. A negative attribute of adenoviral vectors is transient expression, although for appropriate targets this may be a positive attribute. Transient expression may be exacerbated by vector immunogenicity, which can limit multiple cycles of transduction and chronic transgene expression. A second major disadvantage of adenoviral vectors used in vivo is the immune response that can preclude infection and cause the destruction of transduced cells, resulting in local damage and inflammation.

Adenoviruses are nonenveloped, icosahedral, double-stranded (ds) DNA viruses (18). They contain about 36 kb of dsDNA that encodes approximately 30 different mRNA transcripts. This genome is housed in the adenovirus capsid, which consists of three major proteins: hexon (II), penton base (III) and a knobbed fiber (IV). In addition, minor proteins include core proteins and cementing proteins that are important for DNA and capsid interactions. The adenoviral genome can be divided into two main regions, early (E) and late (L), according to the time at which their genes are expressed during virus replication. There are four regions of early genes that are termed E1, E2, E3 and E4, and one region of late genes comprising the five coding units termed L1, L2, L3, L4 and L5. The E1 region is essential for viral replication; therefore, recombinant adenoviruses without the E1 region are considered replication-defective. The E1 region is subdivided into E1A and E1B. The E1A gene product is a viral transcription unit that activates the expression of other adenoviral transcription units by binding to viral promoters. The E1B region codes for a 55-kDa protein that interacts with the cellular p53 tumor suppressor protein. The E2 region codes for viral DNA polymerase and the adenoviral single-stranded (ss) DNA-binding protein. The E3 region is not required for in vitro replication but it does offer the virus some protection against host defense mechanisms. Finally, the E4 region codes for proteins involved in the regulation of viral and cellular protein expression. Removal of genetic material from the vector, such as the E3 and/or the E4 region, allows for larger genes to be inserted and reduces the viral immunogenicity. Viruses without the E3 and E4 region (second-generation vectors) are referred to as "gutless" and have decreased antigenicity. The late genes (L1-L5) are expressed at the onset of viral DNA replication and code for structural polypeptides that are needed for virion assembly. At present, 47 human adenovirus serotypes have been distinguished on the basis of their resistance to neutralization by antisera to other known adenovirus serotypes (19). Ad2 and Ad5 are used primarily for gene therapy applications both in vitro and in vivo.

Contusugene ladenovec (INGN-201, Ad5CMV-p53, Advexin®; Introgen Therapeutics) is an adenovirus (Ad5) in which the E1 region is replaced with the cDNA of the *TP53* gene and is driven by a cytomegalovirus (CMV) promoter (20). Deletion of the E1 region of the parental Ad5 DNA renders contusugene ladenovec a replication-defective virus and prevents the expression of adenoviral genes. Studies with repeated sequencing showed that contusugene ladenovec does not undergo mutational changes, and it maintains wild-type *TP53* DNA throughout the manufacturing process.

There are three different molecular entities that combine an Ad5 vector with a *TP53* expression cassette. Contusugene ladenovec uses a cassette comprising a CMV promoter, a wild-type *TP53* gene and the SV40(A) signal. The Schering-Plough/Canji vector also uses a CMV promoter but has the *PIX* gene deleted from the Ad5 backbone (21). Clinical studies using this vector were discontinued after phase II evaluation in ovarian cancer. In contrast, Gendicine® uses a Rous sarcoma virus (RSV) promoter and BGH(A) tail (22). It was recently approved in China for the treatment of squamous cell head and neck cancer (SCCHN), although this approval has been the subject of much controversy in the U.S.

PRECLINICAL PHARMACOLOGY

Contusugene ladenovec potently inhibited the growth of several human tumor cell lines in vitro, with almost no effect on normal cells (23, 24). The agent has also demonstrated efficacy in nearly all cancer animal models tested, including SCCHN, NSCLC, colorectal and breast cancer (25-28).

The antitumor effects of contusugene ladenovec correlate with exogenous *TP53* expression and induction of apoptosis and decreased proliferation of cells within the tumor (29). Data suggest that inhibition of angiogenesis may contribute to the antitumor effect of Adp53 because overexpression of *TP53* inhibits the expression of vascular endothelial growth factor (VEGF) and *TP53* also increases the expression of thrombospondin-1, which is an inhibitor of angiogenesis (30, 31). Higher levels of p53 protein have been found to inhibit the transcriptional activity of hypoxia-inducible factor (HIF), which is involved in the expression of a number of cell survival factors under hypoxic conditions (32).

Immune responses generated following vector administration may also contribute to effects in tumors. In fact, it has been reported that contusugene ladenovec is able to increase local CD95L expression, which results in massive neutrophil infiltration and tumor growth inhibition (33, 34).

When combined simultaneously in vitro with other cytotoxic agents such as cisplatin, contusugene ladenovec exhibited additive and possibly synergistic antiproliferative activity. In NSCLC, it was shown that wild-type *TP53* transduction of a *TP53*$^{-/-}$ NCI-H358 cell line induced sensitivity to the DNA-damaging drug cisplatin (35). Additive activity was also demonstrated when contusugene ladenovec was combined with radiation therapy. In subcutaneous tumor xenografts, the combination of radiation and wild-type *TP53* transduction increased the percentage of TUNEL-positive cells and showed synergistic effects for tumor growth suppression (36).

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of contusugene ladenovec have been investigated in patients with advanced NSCLC and SCCHN (37, 38). In all these studies contusugene ladenovec was administered intratumorally and detected in plasma, urine, respiratory tract secretions, peripheral blood lymphocytes and feces, but not semen. In plasma and serum, contusugene ladenovec levels varied in a dose- and time-dependent fashion. In particular, Adp53 DNA was detected in blood by polymerase chain reaction (PCR) by 30 min after injection and gradually eliminated over the next 48 h. Cytopathic effect (CPE) assays performed in patients treated at the highest dose levels showed that Adp53 was present in blood at the highest levels 30 min after intratumoral injection, decreased at a rate of 2-4 orders of magnitude by 90 min and further decreased to very low titers by 24 h, to be completely eliminated by 48 h after injection. Adp53 detection in the urine started within 1 day from the beginning of injections. Urine was free of Adp53 within 3-17 days of the last injection. Adp53 was also detected in the sputum within 1 day of injection and was cleared within 7 days.

Another study in 17 patients with advanced cancer administered doses of 3×10^{10} to 3×10^{12} virus particles (vp) for 3 days every 28 days demonstrated detectable Adp53 DNA in plasma in most patients at 14 and 28 days, and 6 patients receiving 1×10^{12} and 3×10^{12} vp had DNA detected in tumor tissue (39).

SAFETY

In the above study, systemic administration was well tolerated, with mild or moderate fever, fatigue, nausea and vomiting. The only unexpected toxicity was a transient elevation in prothrombin time and fibrin degradation products, without evidence of hemorrhage or thrombosis (39).

CLINICAL STUDIES

Several phase I/II or III trials clinical trials involving patients with different types of cancer, including lung, head and neck, prostate and breast cancer and glioblastoma, have been performed to evaluate the safety and biological activity of contusugene ladenovec.

Three phase II trials were conducted in patients with recurrent or advanced SCCHN. The overall objective response rate defined as complete and partial responses was 10% with monotherapy. Tumor growth control defined as stable disease or better was achieved in 59% of all treated lesions. Patients treated with higher doses had a significantly greater median survival than patients treated with lower doses (243 days vs. 119 days). Additionally, the overall median survival was longer in patients who received treatment followed by chemotherapy, suggesting a possible synergy between contusugene ladenovec and recent exposure to chemotherapy (40).

Contusugene ladenovec has also been evaluated in the surgical adjuvant setting in SCCHN (38). Thirty-three patients received multiple intratumoral injections at a dose of 1×10^{11} plaque-forming units (pfu) 3 times a week (this made up 1 course). Patients with resectable tumors received one full course of treatment and two additional administrations followed, one during surgery and one 72 h after surgery. Patients with nonresectable tumors received treatment every 4 weeks. The treatment regimen was well tolerated, the most common adverse effect being injection-site pain, which did not seem to be related to the dose or the anatomic site of injection. Other common side effects included transient fever, headache, pain and edema; these symptoms mainly occurred at doses above 10^{10} pfu. No allergic reactions or evidence of systemic hypersensitivity were noted. Patients with resectable (recurrent disease) versus nonresectable disease were analyzed separately. Of the nonresectable arm, 2 of 17 patients showed a partial response, 6 had stable disease for 1-3.5 months and 9 had progressive disease. Of the resectable arm, 4 of 15 patients remained free of disease with a median follow-up of 18 months.

Contusugene ladenovec has also been used in chemoprevention as a mouthwash preparation for patients with premalignant oral lesions in order to evaluate whether it can slow the progression of premalignant lesions to a cancerous state (41, 42).

A phase I study was performed in patients with advanced NSCLC by Fujiwara et al. (37). Fifteen patients with advanced NSCLC who had failed prior conventional therapies were enrolled in this study. Three patients were treated with contusugene ladenovec as monotherapy at each dose level, and if no toxicity was observed with the vector then the next three patients received contusugene ladenovec in combination with i.v. cisplatin (80 mg/m^2). In total, nine patients have been treated with contusugene ladenovec as monotherapy and six patients received the gene therapy in combination with cisplatin. Thirteen of 15 patients (86.7%) were assessable for response. Objective responses included a partial response in 1 patient (7.7%), stable disease in 10 patients (76.9%) and progressive disease in 2 patients (15.4%). Among the 15 treated patients, none withdrew from the study as a result of toxicity, and no grade 4 toxicity was observed. The most frequent vector-related adverse event was transient fever.

Another clinical trial testing the combination of contusugene ladenovec and chemotherapy was performed in patients with NSCLC. In this phase I trial 24 NSCLC patients were treated with cisplatin and contusugene ladenovec (43). Cisplatin was administered i.v. and 3 days later the gene therapy was delivered by intratumoral injection. Up to a total of six monthly courses were administered. Seventeen patients remained stable for at least 2 months, 2 achieved partial responses, 4 continued with progressive disease and 1 patient was not evaluable due to progressive disease. When tumor biopsies were analyzed for apoptosis, 14% demonstrated no change, 7% showed a decrease in apoptosis and 79% demonstrated an increased number of apoptotic cells. Cisplatin alone has been shown to induce apoptosis to a small degree, but contusugene ladenovec or its combination with cisplatin showed significantly greater killing. Despite these preliminary results, no studies have shown that the addition of gene therapy to chemotherapy is better than chemotherapy alone in NSCLC patients. A multicenter phase II trial comparing groups of patients receiving adenoviral *TP53* gene therapy (Sch-58500) plus chemotherapy versus chemotherapy as a first-line treatment showed no differences in response rate and survival benefit between the two groups (44).

A phase II clinical trial of contusugene ladenovec in conjunction with radiation therapy was carried out in 17 patients with localized inoperable NSCLC too ill to receive chemotherapy (45). The overall response rate was 29% (5 of 17 patients) and the response rate at the local injection site was 52.9% (9 of 17 patients). The survival rate at 1

year was 56%. Posttreatment biopsies of the original tumor site were obtained 3 months after completion of treatment. In 12 cases the biopsy showed no evidence of tumor. This biopsy-negative rate of 70% compares favorably with that of 17% reported in studies of chemotherapy combined with radiation therapy, suggesting that the interaction of contusugene ladenovec and radiation therapy may potentially improve local tumor control. Safety data indicated that this combination had an acceptable safety profile. Thirteen patients underwent 61 computed tomography-guided biopsies or drug administrations. Thirteen (21%) resulted in pneumothorax, 1 of which required hospital admission. Six of the 17 patients experienced a grade 3 or 4 adverse event.

An interesting experience exploring contusugene ladenovec combined with chemotherapy was reported in patients with locally advanced breast cancer (46). Patients were treated with intratumoral injections of contusugene ladenovec on days 1 and 2 plus docetaxel 75 mg/m^2 i.v. and doxorubicin 50 mg/m^2 i.v. on day 1, as well as prophylactic granulocyte colony-stimulating factor (G-CSF). Thirteen patients were enrolled with a median age of 56 years (range = 39-71 years) and a median tumor size of 8.0 cm (range = 5-11 cm). Up to six cycles of contusugene ladenovec were given and all patients underwent surgery. Eight patients (83%) had *TP53* mutations. The toxicity profile for contusugene ladenovec plus chemotherapy was similar to results obtained for the gene therapy alone. No additional toxicity to chemotherapy was observed and no grade 3 side effects that occurred were considered to be related to contusugene ladenovec. Twelve patients were evaluated for response. None of the 12 evaluated patients achieved a complete pathological response, but all evaluated patients achieved a partial clinical response and underwent surgery. Eight patients (67%) had residual pathological foci of disease in the breast inferior to 10 mm. The mean size of the residual tumor in the breast was 1.78 cm. All specimens showed extensive T-lymphocyte infiltrates (CD3 80%, CD4 30%, CD8 70%). A retrospective, exploratory analysis was performed in patients with locally advanced breast cancer who were treated under The University of Texas M.D. Anderson Cancer Center (MDA) protocol MDA ID-97099. The objectives of the analysis were to describe and compare the clinical and pathological response in size- and volume-matched patients who were treated with a combination of docetaxel and doxorubicin versus a group of patients who received the same regimen with local injection of contusugene ladenovec. Twenty-two consecutive patients were collected who had an initial presentation that more closely matched the initial presentation of the 12 patients who received combined treatment. The historical comparison demonstrated that there was a significantly improved clinical response with the combination regimen (41% vs. 100%; $P = 0.0006$). These data demonstrate that combination of contusugene ladenovec/docetaxel/doxorubicin has significant therapeutic activity and could represent an interesting approach in the treatment of primary breast cancer.

The North American Brain Tumor Consortium (NABTC) completed a phase I trial of adenovirus-mediated *TP53* gene therapy in recurrent human malignant glioma. Fifteen patients with recurrent glioblastoma were treated using a two-stage approach (47). Contusugene ladenovec was injected intratumorally via a stereotactically implanted catheter, after which the tumor and catheter were resected en bloc and reinjections were performed into the walls of the tumor cavity. Increased apoptosis mediated by transactivation of p21 was observed. Clinical toxicity was minimal and no signals of systemic viral dissemination were detected. Median overall survival was 10 months and 1 patient with glioblastoma remained free of recurrence for more than 3 years.

Two ongoing trials are comparing the safety, efficacy and overall survival on treatment with contusugene ladenovec as monotherapy or in combination with chemotherapy in patients with head and neck cancer. In one of these trials (T301) (48), patients with local or regional recurrent, refractory SCCHN who had failed radiation therapy and chemotherapy with platinum-containing drugs or taxanes were randomized to either contusugene ladenovec intratumorally or methotrexate intravenously. Patients were treated for a maximum of 9 cycles (27 weeks). Survival is the primary endpoint of the study and predicted accrual to accomplish this endpoint is 240 patients. In the second study (T302) (49), patients with local or regional recurrent SCCHN who have not been previously exposed to chemotherapy are randomized to receive a combination of intratumoral contusugene ladenovec, cisplatin and 5-fluorouracil (5-FU) or standard care with cisplatin/5-FU. The primary endpoint is time to progression and predicted accrual to accomplish this endpoint is 288 patients.

Contusugene ladenovec has been submitted for approval for the treatment of Li-Fraumeni syndrome (LFS) (50), a genetic disorder that greatly increases the risk of developing several types of cancer, particularly at a young age. The majority of LFS families have *TP53* mutations. Contusugene ladenovec therapy has been successfully used on a compassionate-use basis under a protocol accepted by the FDA. Based upon its initial findings, Introgen has decided to continue to make the gene therapy available on a compassionate-use basis to qualified LFS patients through physician-sponsored protocols at qualifying institutions. As a result of the success in treating LFS with contusugene ladenovec, Introgen's subsidiary Gendux Molecular submitted an Orphan Drug Designation Request to the EMEA for the use of the gene therapy to treat LFS, which was subsequently granted.

CONCLUSIONS

Clinical evaluation of contusugene ladenovec has been reported in various tumor types using different administration schedules. A significant amount of information has emerged during the last 10 years, especially in head and neck cancer (both as monotherapy and in combination with chemotherapy), NSCLC (combined with radiation therapy) and breast cancer (combined with chemotherapy). As a result of these experiences, it appears that contusugene ladenovec has an excellent safety profile and does not enhance the toxicity of either chemotherapy or radiation, making it a good candidate for combination treatment strategies. Moreover, contusugene ladenovec either alone or in combination with chemotherapy may enhance locoregional control in head and neck cancer. Current treatment modalities for recurrent head and neck cancer are suboptimal. Loco-regional control of recurrent disease has been shown to offer benefit with respect to morbidity in patients with head and neck cancer, although little survival advantage has been demonstrated. Results of the ongoing clinical trials T301 and T302 are eagerly awaited. Finally, the most powerful and intriguing use of contusugene ladenovec may ultimately be in cancer prevention programs.

SOURCE

Introgen Therapeutics, Inc. (US).

REFERENCES

1. Vogelstein, B., Lane, D., Levine, A.J. *Surfing the p53 network*. Nature 2000, 408(6810): 307-10.
2. Isobe, M., Emanuel, B.S., Givol, D., Oren, M., Croce, C.M. *Localization of gene for human p53 tumour antigen to band 17p13.* Nature 1986, 320(6057): 84-5.
3. Kastan, M.B., Cancan, C.E., Leonard, C.J. *p53, cell cycle control and apoptosis: Implications for cancer.* Cancer Metastasis Rev 1995, 14(1): 3-15.
4. Prives, C., Hall, P.A. *The p53 pathway*. J Pathol 1999, 187(1): 112-26.
5. El Deiry, W.S. *Regulation of p53 downstream genes*. Semin Cancer Biol 1998, 8(5): 345-57.
6. Miyashita, T., Reed, J.C. *Tumor suppressor p53 is a direct transcriptional activator of the human bax gene.* Cell 1995, 80(2): 293-9.
7. Enock, T., Norbury, C. *Cellular responses to DNA damage: Cell-cycle checkpoints, apoptosis and the role of p53 and ATM.* Trends Biol Sci 1995, 20(10): 426-31.
8. Dameron, K., Volpert, O.V., Tainsky, M.A., Bouck, N. *Control of angiogenesis in fibroblast by p53 regulation of thrombospondin-1.* Science 1994, 265(5178): 1582-4.
9. Xiong, Y., Hannon, G.J., Zhang, H., Casso, D., Kobayashi, R., Beach, D. *P21 is a universal inhibitor of cyclin kinases*. Nature 1993, 366(6456): 701-4.
10. Harper, J.W., Adami, G.R., Wei, N., Keyomarsi, K., Elledge, S.J. *The p21 CDK-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases.* Cell 1993, 75(4): 805-16.
11. Hollstein, M., Sidransky, D., Vogelstein, B., Harris, C.C. *p53 mutations in human cancers*. Science 1991, 253(5015): 49-53.
12. Quinlan, D.C., Davidson, A.G., Summers, C.L., Warden, H.E., Doshi, H.M. *Accumulation of p53 protein correlates with a poor prognosis in human lung cancer*. Cancer Res 1992, 52(17): 4828-31.
13. Thor, A.D., Moore, D.H. II, Edgerton, S.M. et al. *Accumulation of p53 tumor suppressor gene protein: An independent marker of prognosis in breast cancers*. J Natl Cancer Inst 1992, 84(11): 845-55.
14. Soussi, T. *p53 mutations and resistance to chemotherapy*. Cancer Cell 2003, 3(4): 303-5.
15. Peng, K.W., Vile, R. *Vector development for cancer gene therapy*. Tumor Targeting 1999, 4: 3-11.
16. Roth, J.A., Nguyen, D., Lawrence, D.D. et al. *Retrovirus-mediated wildtype p53 gene transfer to tumors of patients with lung cancer*. Nat Med 1996, 2(9): 985-91.
17. Russell, W.C. *Update on adenovirus and its vectors*. J Gen Virol 2000, 81(Pt. 11): 2573-604.
18. Barnett, B.G., Crews, C.J., Douglas, J.T. *Targeted adenoviral vectors*. Biochim Biophys Acta 2002, 1575(1-3): 1-14.
19. Douglas, J.T. *Adenoviral vectors for gene therapy.* Mol Biotechnol 2007, 36(1): 71-80.
20. *INGN 201: Ad-p53, Ad5CMV-p53, adenoviral p53, p53 gene therapy—introgen, RPR/INGN 201.* Drugs R D 2007, 8(3): 176-87.
21. Wilson, J.M. *Gendicine: The first commercial gene therapy product*. Hum Gene Ther 2005, 16(9): 1014-5.
22. Barnard, D.L. *Technology evaluation: Sch-58500, Canji.* Curr Opin Mol Ther 2000, 2(5): 586-92.
23. Shono, T., Tofilon, P.J., Schaefer, T.S., Parikh, D., Liu, T.J., Lang, F.F. *Apoptosis induced by adenovirus-mediated p53 gene transfer in human glioma correlates with site-specific phosphorylation*. Cancer Res 2002, 62(4): 1069-76.
24. Wang, J., Bucana, C.D., Roth, J.A., Zhang, W.W. *Apoptosis induced in human osteosarcoma cells is one of the mechanisms for the cytocidal effect of Ad5CMV-p53*. Cancer Gene Ther 1995, 2(1): 9-17.
25. Liu, T.J., Zhang, W.W., Taylor, D.L., Roth, J.A., Goepfert, H., Clayman, G.L. *Growth suppression of human head and neck cancer cells by the introduction of a wild-type p53 gene via a recombinant adenovirus*. Cancer Res 1994, 54(14): 3662-7.
26. Spitz, F.R., Nguyen, D., Skibber, J.M., Cusack, J., Roth, J.A., Christiano, R.J. *In vivo adenovirus-mediated p53 tumor suppressor gene therapy for colorectal cancer.* Anticancer Res 1996, 16(6B): 3415-22.
27. Seth, P., Katayose, D., Li, Z. et al. *A recombinant adenovirus expressing wild type p53 induces apoptosis in drug-resistant human breast cancer cells: A gene therapy approach for drug-resistant cancers.* Cancer Gene Ther 1997, 4(6): 383-90.
28. Zhang, W.W., Fang, X., Mazur, W., French, B.A., Georges, R.N., Roch, J.A. *High efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus*. Cancer Gene Ther 1994, 1(1): 5-13.
29. Harris, M.P., Sutjipto, S., Wills, K.N. et al. *Adenovirus-mediated p53 gene transfer inhibits growth of human tumor cells expressing mutant p53 protein*. Cancer Gene Ther 1996, 3(2): 121-30.
30. Bouvet, M., Ellis, L.M., Nishizaki, M. et al. *Adenovirus-mediated wildtype p53 gene transfer downregulates vascular endothelial growth factor expression and inhibits angiogenesis in human colon cancer.* Cancer Res 1998, 58(11): 2288-92.
31. Nishizaki, M., Fujiwara, T., Tanida, T. et al. *Recombinant adenovirus expressing wild-type p53 is antiangiogenic: A proposed mechanism for bystander effect.* Clin Cancer Res 1999, 5(5): 1015-23.
32. Blagosklonny, M.V., Giannakakou, P., Romanova, L.Y., Ryan, K.M., Vousden, K.H., Fojo, T. *Inhibition of HIF-1 and wild-type p53-stimulated transcription by codon Arg175 p53 mutants with selective loss of functions*. Carcinogenesis 2001, 22(6): 861-7.
33. Waku, T., Fujiwara, T., Shao, J. et al. *Contribution of CD95 ligand-induced neutrophil infiltration to the bystander effect in p53 gene therapy for human cancer*. J Immunol 2000, 165(10): 5884-90.
34. Miller, G., Lahrs, P.V.G., Shah, A.B., DeMatteo, R.P. *Adenovirus infection enhances dendritic cell immunostimulatory properties and induces natural killer and T cell mediated tumor protection*. Cancer Res 2002, 62(18): 5260-6.
35. Fujiwara, T., Grimm, E.A., Mukhopadhyay, T. et al. *Induction of chemosensitivity in human lung cancer cells in vivo by adenoviral-mediated transfer of the wild-type p53 gene*. Cancer Res 1994, 54(15): 2287-91.
36. Nishizaki, M., Meyn, R.E., Levy, L.B. et al. *Synergistic inhibition of human lung cancer cell growth by adenovirus-mediated wild-type p53 gene transfer in combination with docetaxel and radiation therapeutics in vitro and in vivo.* Clin Cancer Res 2001, 7(9): 2887-97.
37. Fujiwara, T., Tanaka, N., Kanazawa, S. et al. *Multicenter phase I study of repeated intratumoral delivery of adenoviral p53 in patients with advanced non–small-cell lung cancer*. J Clin Oncol 2006, 24(11): 1689-99.

38. Clayman, G.L., el-Naggar, A.K., Lippman, S.M. et al. *Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma*. J Clin Oncol 1998, 16(6): 2221-32.

39. Tolcher, A.W., Hao, D., de Bono, J. et al. *Phase I, pharmacokinetic, and pharmacodynamic study of intravenously administered Ad5CMV-p53, an adenoviral vector containing the wild-type p53 gene, in patients with advanced cancer.* J Clin Oncol 2006, 24(13): 2052-8.

40. Gabrilovich, D. *INGN 201 (Advexin): Adenoviral p53 gene therapy for cancer*. Expert Opin Biol Ther 2006, 6(8): 823-32.

41. *Gene therapy in preventing cancer in patients with premalignant carcinoma of the oral cavity of pharynx (NCT00064103).* ClinicalTrials.gov Web site, March 2, 2009.

42. *Wild type p53 adenovirus for oral premalignancies (NCT00410865).* ClinicalTrials.gov Web site, March 2, 2009.

43. Nemunaitis, J., Swisher, S.G., Timmons, T. et al. *Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small cell lung cancer*. J Clin Oncol 2000, 18(3): 609-22.

44. Schuler, M., Herrmann, R., De Greve, J. et al. *Adenovirus-mediated wild-type p53 gene transfer in patients receiving chemotherapy for advanced non-small-cell lung cancer: Results of a multicenter phase II study.* J Clin Oncol 2001, 19(6): 1750-8.

45. Swisher, S.G., Roth, J.A., Komaki, R. et al. *Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy.* Clin Cancer Res 2003, 9(1): 93-101.

46. Cristofanilli, M., Krishnamurthy, S., Guerra, L. et al. *A nonreplicating adenoviral vector that contains the wild-type p53 transgene combined with chemotherapy for primary breast cancer: Safety, efficacy, and biologic activity of a novel gene-therapy approach*. Cancer 2006, 107(5): 935-44.

47. Lang, F.F., Bruner, J.M., Fuller, G.N. et al. *Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: Biological and clinical results.* J Clin Oncol 2003, 21(13): 2508-18.

48. *Study to compare the overall survival of patients receiving INGN 201 (study drug) with patients receiving methotrexate (NCT00041613).* ClinicalTrials.gov Web site, March 2, 2009.

49. *Effectiveness and safety of INGN 201 in combination with chemotherapy versus chemotherapy alone (NCT00041626).* ClinicalTrials.gov Web site, March 2, 2009.

50. Senzer, N., Nemunaitis, J., Nemunaitis, M. et al. *p53 therapy in a patient with Li-Fraumeni syndrome.* Mol Cancer Ther 2007, 6(5): 1478-82.