6.1. In Vivo Efficacy Study

6.1.1 Description of the Methods

This study was performed in 8-10 week old female SCID mice. Mice were housed in individually ventilated cages and given pelleted food (Teklad Lab Animal Diets, Harlan Laboratories, USA) and water ad libitum in a temperature (25±3°C) and humidity (50–70%) controlled environment with a 12-h/12-h dark-light cycle. All animal experimentations were carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines, using (IAEC) Institutional Animal Ethics Committee approved protocols in an AAALAC International (Association for Assessment and Accreditation of Laboratory Animal Care International) accredited facility which complies with the National Institutes of Health guidelines for care and use of animals.(1–3)

SCID mice were injected subcutaneously on the right flank with cultured A549 (5×10^6 cells in 200 µL of RPMI 1640 with 33% Matrigel [BD Biosciences, San Jose, CA]. When xenograft tumor volumes, reached 150 mm^3 (group mean), mice were divided into four groups (n=6) and received the following agents either alone or in combination as specified by the experimental protocols: vehicle or Taxotere® (20mg/kg), docetaxel nano particle (20mg/kg) or cetuximab conjugated docetaxel nanoparticle once weekly (QW) via lateral tail vein. Tumor dimensions were assessed twice weekly using an electronic digital caliper. Tumor volume was calculated as (width2 × length/2). Body weight was recorded twice weekly as an index of toxicity.(4,4,5)

6.3. Results and Discussion

To demonstrate the antitumor efficacy, Taxotere®, Docetaxel NPs and Cetuximab Conjugated Docetaxel NPs were injected in human lung cancer-bearing mice via lateral tail vein. In animals bearing established A549 tumors, docetaxel (Taxotere® 20 mg/kg, QW), a standard-of-care chemotherapy agent in NSCLC, significantly inhibited tumor growth compared with vehicle.(6–9) Treatment with Taxotere caused 60.3% inhibition in tumor growth on day 21 compared to vehicle control. Similar to Taxotere similar dosing regimen of docetaxel nano particles showed significant reduction in tumor growth compared to vehicle control. When mice were injected with 20 mg/Kg Docetaxel NPs, demonstrated an enhanced antitumor efficacy compared to the Docetaxel alone. The anti-tumor effect of docetaxel nanoparticle was associated with the body

weight loss may be due to the availability or tissue penetration in the different tissues. However, when combined at the same dose with cetuximab exerts significantly greater antitumor effect than that observed with single agent alone.(4)(4,10,11) Treatment with docetaxel NPs caused 72.5% reduction on tumor volume while 81% inhibition was observed in cetuximab-conjugated docetaxel NPs. Surprisingly anti-tumor effect was associated with lack of body weight loss in the cetuximab-conjugated docetaxel NPs indicating that the drug was targeted to site of action. Thus, Nano-sized docetaxel can provide advantages of reducing the high dose dependent toxicity of anticancer drugs while, at the same time increasing their anticancer efficacy.(4,12,13) Furthermore, this result clearly indicate that antibody (Cetuximab) conjugation to Docetaxel NPs is successful in inhibiting tumor growth while lower toxicity effect.
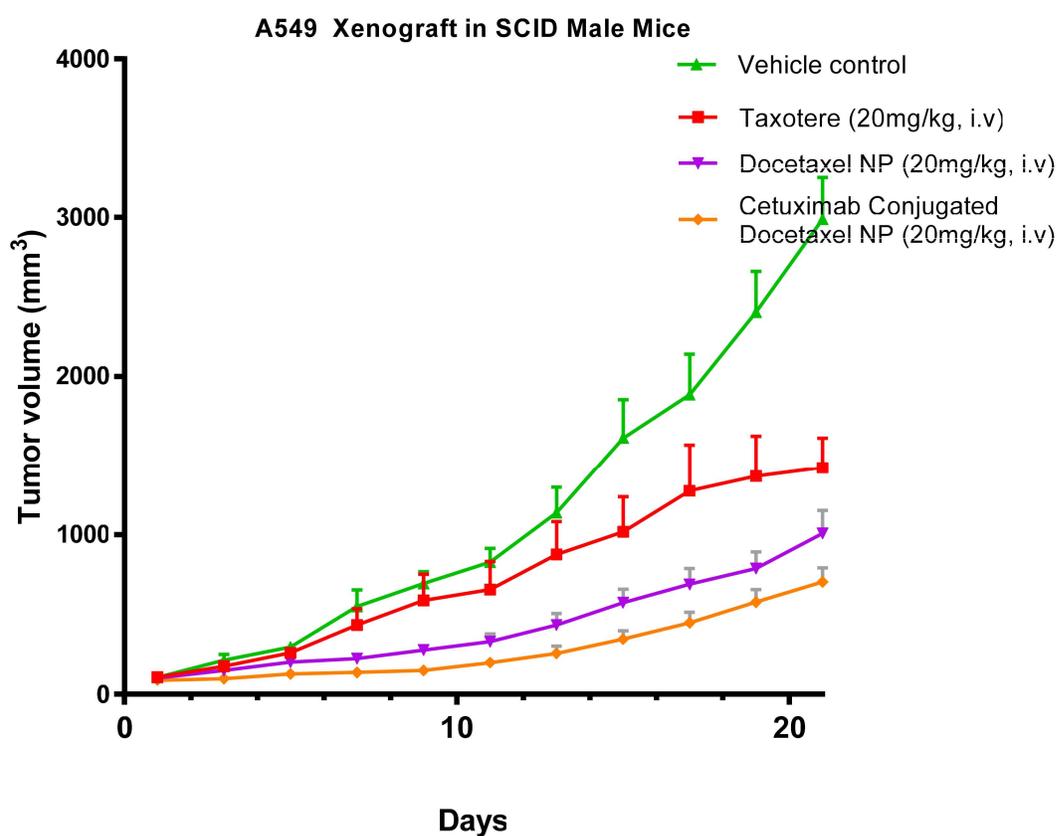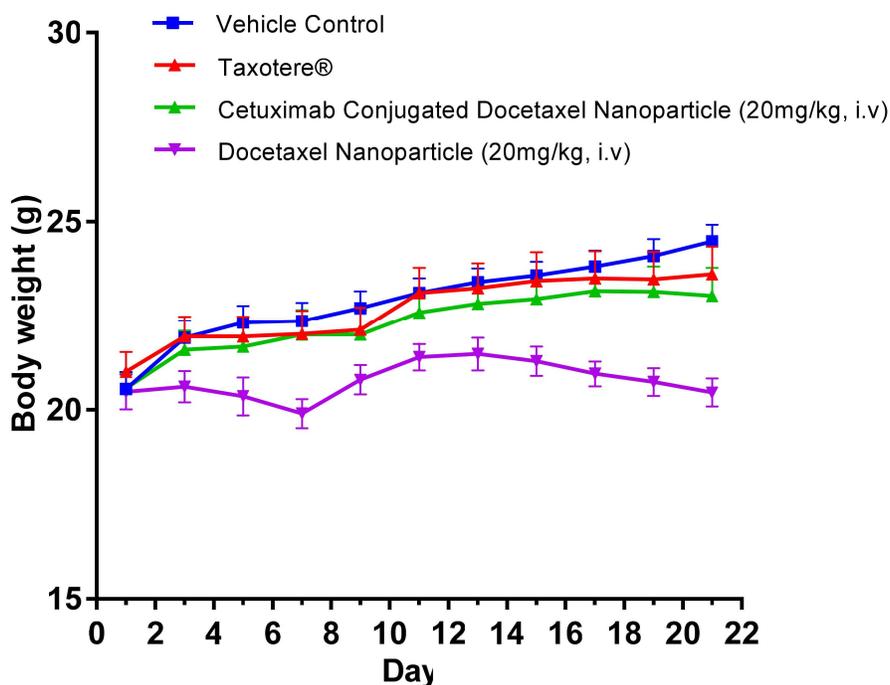

Figure 6.1

A549 Xenograft model: Tumor volume vs Days

A549 Xenograft in SCID Male Mice

Figure 6.2 A549 Xenograft model: Body weight vs Days

Conclusions

Our data show that docetaxel nanoparticles exert a strong antitumor activity over marketed docetaxel in A549 NSCLC subcutaneous xenograft model. When conjugated with cetuximab with docetaxel nanoparticle was significantly greater than docetaxel alone treatment in the xenograft models. Toxicity was significantly reduced in docetaxel nanoparticles conjugated with cetuximab compared to docetaxel nanoparticle alone.

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