ABSTRACT SUMMARY
Heat shock protein (HSP90) is a validated target for cancer treatment. Geldanamycin (GDM) is a potent HSP90 inhibitor. However, its clinical applications are limited due to dose-limiting toxicities and limited water solubility. Here we report a water soluble polymeric conjugate of GDM that has the potential for clinical application. The conjugate is expected to release GDM only at tumor sites due to the use of a linker that is recognized by the enzyme cathepsin B which is overexpressed at tumor sites. We report that the conjugation of GDM to degradable peptidic dendrimer maintains its antiproliferative properties. We also report the potential of degradable water soluble PEGylated-GDM conjugate for prostate cancer treatment.

INTRODUCTION
Prostate cancer is the second leading cause of cancer death in American men. Current therapies based on androgen deprivation frequently leads to castrate-resistant prostate cancer (CRPC). New strategies are required for CRPC treatment.

Hsp90α is the most abundant heat shock protein within the cell. Client proteins of HSP90α impact an array of functions that affect health and disease, including known oncoproteins, which makes HSP90 an anti-cancer target. Geldanamycin (GDM) has been studied as anticancer agent due to its capacity to inhibit HSP90. However, the clinical use of GDM is limited by high hepatotoxicity, low solubility in water, and metabolic instability.

Conjugation of GDM to polymers can increase the water solubility, minimize drug degradation and keep the drug inactive until it is released at tumor sites. However, the clearance of the nondegradable polymeric backbone is an issue. Several preclinical studies are raising concerns about biosafety of nondegradable polymers after chronic administration.

One strategy for achieving polymer degradation and drug release from the carrier is to use an enzymatically degradable linker. Previously we have demonstrated that peptidic dendrimers prepared using tetrapeptide GFLG are completely degraded in the presence of enzyme cathepsin B. In this study we demonstrate the potential of the peptidic dendrimer and its PEGylated analog for the delivery of GDM.

EXPERIMENTAL METHODS
Chemistry: GDM was reacted with diaminohexane to obtain AHGDM which was conjugated to the tetrapeptide GFLG. GFLGAHGDM was then reacted with tetrakis(p-nitrophenyl) ester of ethylenediaminotetraacetic acid (EDTA) to obtain GDM containing peptidic dendrimer. Sequential addition of GFLGAHGDM and GFLG-PEG to the central core yielded degradable water-soluble system whereas sequential addition of AHGDM and PEG yielded nondegradable water-soluble system.

Stability and Drug Release Studies: To determine the stability, conjugates were incubated with PBS, FBS or acetate buffer. Drug release in presence of cathepsin B was determined after incubation of polymeric conjugates with the enzyme.

Growth Inhibition Assay: WST (watersoluble tetrazolium salt) assay was used to determine the antiproliferative effect of GDM containing conjugates using androgen independent prostate cancer cell line (DU145).

RESULTS AND DISCUSSION
Design and schematic representation of water soluble degradable and nondegradable GDM containing conjugates and a four arm
peptidic dendrimer is shown in Fig 1. All the conjugates were characterized using NMR, MALDI and LCMS to determine purity and drug content. Molecular weight and drug content of these conjugates are shown in Table 1.

![Fig. 1 Structure of the GDM conjugates. A. Four arm degradable dendrimer. B. Degradable PEGylated GDM. C. Non degradable PEGylated GDM.](image)

**Table 1. Characteristics of GDM conjugates**

<table>
<thead>
<tr>
<th>Compound</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;, Calc.</th>
<th>M&lt;sub&gt;n&lt;/sub&gt;, MALDI</th>
<th>% AHGDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A EDTA(GFLG)AHGDM&lt;sub&gt;4&lt;/sub&gt;</td>
<td>4294.34</td>
<td>4320</td>
<td>60.1%</td>
</tr>
<tr>
<td>B PEG-GFLG-AHGDM</td>
<td>4685.91</td>
<td>4674.63</td>
<td>13.7%</td>
</tr>
<tr>
<td>C PEG-EDTA-AHGDM</td>
<td>3919.03</td>
<td>3926.24</td>
<td>16.4%</td>
</tr>
</tbody>
</table>

Peptidic dendrimer A was completely stable in PBS, FBS (10 % in PBS) and acetate buffer up to 48h (fig 2B) in presence of cathepsin B.

A sustained release of the drug in form of either AHGDM or glycine-AHGDM (fig 2C), was observed suggesting 2 main sites of degradation by the enzyme (fig 2A). Collectively approximately 30% of the drug was released within 48h.

![Fig 2. Stability and Drug Release Profile. A. Scheme of drug release. B. Stability in presence of acetate buffer, PBS or 10% serum. C. Drug release profile after incubation with Cathepsin B.](image)

The ability of GDM conjugates to inhibit the growth of DU-145 cells was evaluated after incubation for 48, 72 and 96h. GI<sub>50</sub> values are reported in Table 2. Consistent with drug release profile, a decrease in GI<sub>50</sub> values was observed with the increase in the incubation time. After 96h treatment GI<sub>50</sub> value for A was similar to the GI<sub>50</sub> value of AHGDM. Conjugation of PEG to the core increased water solubility, albeit with loss in activity. However, GI<sub>50</sub> value of degradable PEGylated GDM was still in the micromolar range. GI<sub>50</sub> values of non degradable PEGylated GDM were 3-4 fold higher than its degradable counterpart suggesting the importance of degradable linker.

![Table 2. GI<sub>50</sub> values of Geldanamycin, AHGDM and drug conjugates after incubation with cell line DU145](image)

**CONCLUSION**

A simple water soluble GDM conjugate can release the drug only in presence of cathepsin B and has the potential to be used as delivery vehicle for geldanamycin.

**REFERENCES**

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