Presenter Disclosure:
John Nemunaitis, MD

The following relationships exist with this disclosure: Investigator for clinical study sponsored by ZIOPHARM Oncology Inc
Nonclinical and Phase I Clinical Studies with a Regulated Adenoviral Gene Delivery of IL-12 Show Promising Clinical Activity in Unresectable Stage III/IV Melanoma

John Nemunaitis MD, Mary Crowley Cancer Research Centers
Preclinical Background: Regulatable Gene Therapy Strategy

- A replication-deficient adenoviral vector (INXN-2001) engineered to express IL-12 under the control of the RheoSwitch Therapeutic System® (RTS®) gene switch platform (Intrexon Corp / ZIOPHARM Oncology, Boston) was administered via intratumoral injection.
- Activator ligand (INXN-1001) demonstrates well tolerated toxicology at oral dose.
- IL-12 protein levels produced in the tumor correlated with the dose of INXN-1001 in mouse models.
- Antitumor response in syngeneic tumor models was dose-dependent and correlated with dose escalation of INXN-1001.
RheoSwitch® Platform in the Presence of Activator Ligand Controls Timing and Level of Target Gene Expression

**Gene OFF**

- RheoSwitch®
  - VP16-RXR

**Gene ON (+ oral activator ligand)**

- RheoSwitch®
  - Gal4-EcR
- Basal Transcription Proteins
- Co-activator protein
- AL
- Inducible Promoter
- Target Gene

Inducible Promoter Target Gene
IL-12 Production is Modulated by Activator Ligand in HT 1080 Cells
Ad-RTS-mIL-12 + Oral Activator Ligand Induces Systemic Tumor Response in B16 Mouse Melanoma Model

Group 1 untreated; Group 2 AL in food ~675 mg/m²/day; Group 3 Ad-RTS-mIL-12 1x10¹⁰ vp Days 12 & 19; Group 4 AL + Ad-RTS-mIL-12. Arrows = administration of Ad-RTS-mIL-12
INXN-1001 + Ad-RTS-mIL-12 increases CD8+ TILs in the 4T1 Syngeneic Mouse Model
A Phase I/II, Open Label Study of Ad-RTS-hIL-12, an Adenovirus Vector Engineered to Express hIL-12, in Combination with an Oral Activator Ligand, in Subjects with Unresectable Stage III or IV Melanoma

- Phase I, 3+3 dose escalation
- Subjects with unresectable stage III/IV melanoma
- Intratumoral injection of $10^{12}$ viral particles (INXN-2001) on Day 1 of each cycle
- INXN-1001 (5, 20, 100, or 160 mg) on Days 1-7 of each cycle
- Up to six 21-day cycles

**Primary endpoint** ➔ Safety and tolerability of intratumoral injections of $10^{12}$ vp Ad-RTS-hIL-12 in combination with escalating doses of INXN-1001

**Secondary endpoint** ➔ Determine correlation of IL-12 protein with toxicity/response

### Demographics

<table>
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<th>Race</th>
<th>n (%)</th>
<th>Age (years)</th>
<th>Gender n (%)</th>
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<tbody>
<tr>
<td>Caucasian</td>
<td>11 (91)</td>
<td>Mean 65.4</td>
<td>Female 6 (50)</td>
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<tr>
<td>Black</td>
<td>0</td>
<td>Range 33-70</td>
<td>Male 6 (50)</td>
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<td>Hispanic</td>
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<tr>
<td>Pacific Islander</td>
<td>1 (9)</td>
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Biological & Clinical Activity of Ad-RTS-hIL-12

• Biological activity observed at all doses of INXN-1001, in a dose dependent fashion
  – Elevation in serum IL-12 correlated with IFN-γ elevation

• Dose response clinical activity: observed only in subjects treated at higher doses of INXN-1001 (100 mg and 160 mg)

• Clinical activity correlates with high levels of IL-12, IFN-γ production
Diverse Patterns of Clinical Activity

- Initial increase in size of injected and non-injected lesions due to inflammatory response in a subset of subjects
- Tumor disappearance, or flattening in 3/6 (50%) subjects treated at the 100 and 160 mg doses of INXN-1001
- Decrease in size in one subject at the 160 mg dose
- One subject with progressive disease with prior therapy completed 18 weeks of therapy without clinical deterioration
Prominent Inflammatory Response Correlates with High levels of IFN-γ

- Initial increase in lesion size due to inflammatory response seen at Cycle 1 Day 16
- Lesion was undetectable at Cycle 2 Day 1
- Subject ultimately progressed and was taken off study
Serum IL-12 Levels Across Treatment Cycles

7.4 ng/mL = $C_{\text{max}}$ of systemically delivered IL-12 at the MTD (0.5mg/kg)
Clinical Activity Observed in Subjects with the Highest Levels of IFN-γ

Serum IFN-γ Levels

Completed 6 Cycles (18 weeks)
Changes in Relevant Immune Cell Populations Trend in a Favorable Direction in Subjects in Higher dose Cohorts of INXN-1001

<table>
<thead>
<tr>
<th></th>
<th>Absolute CD8+ (Cytotoxic T Cells)</th>
<th>Absolute CD3+ (T Cells)</th>
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<tbody>
<tr>
<td></td>
<td>Screening</td>
<td>C2D15</td>
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<tr>
<td><strong>Median Cell Number for Low Doses of INXN-1001</strong></td>
<td>283</td>
<td>430.5</td>
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<tr>
<td><strong>Median Cell Number for High Doses of INXN-1001</strong></td>
<td>435.5</td>
<td>383</td>
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</table>
Safety Summary

• Most common (≥20% of subjects) TEAEs by Preferred term
  Chills, Fatigue and Pyrexia (73.3% each), Nausea (66.7%), Anorexia and Anemia (40% each), Vomiting (33.3%) and Diarrhea, Peripheral edema, Hyponatremia, Arthralgia, Pain in extremity, Dizziness, Headache, and Pruritus (20% each).

• Most common ((≥20% of subjects) related TEAEs by Preferred term
  Chills and Pyrexia (73% each), Nausea (67%), Fatigue (60%), Vomiting (33%), Anorexia (27%), Arthralgia and Diarrhoea (20% each).

• ≥ Grade 3 TEAEs
  Hyponatremia, Fatigue, Pyrexia, Aspartate aminotransferase increased, White blood cell count decreased, and Dehydration were assessed as related to study drug by the investigator. All other TEAEs in this category were assessed as unrelated to study drug by the investigator.

• One death secondary to septicemia, unrelated to study drug
Summary of Immunological and Clinical Activity

- Clinical activity at 100 mg and 160 mg doses coincided with the highest serum levels of IL-12 and IFN-γ
  - 4-fold median increases from baseline at peak levels compared with lower dose cohorts

- Flow cytometric analyses of PBMCs at 100 mg and 160 mg doses revealed
  - 7-fold and 4-fold median increase from baseline at peak levels in absolute CD3+ and CD8+ T cell values, respectively, compared with lower dose cohorts

- Clinical activity at higher dose cohorts
  - Prominent inflammatory responses in injected and non-injected lesions
  - Transient decrease in size of injected and non-injected lesions
Conclusion

- Combination of INXN-2001 and INXN-1001 was well tolerated in advanced melanoma
- Evidence of immune response with correlation of IL-12 and IFN-γ induction was demonstrated
- Phase II testing is justified