

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults (Gittleman, 2018). With intensive research efforts and a multimodal management that consists of surgery, radiotherapy and chemotherapy with temozolomide, the prognosis remains very poor with 5-year survival rate of less than 10% (Kamiya-Matsuoka, 2015). Therefore, there is an unmet medical need to develop novel treatments for GBM.

It has been demonstrated that *ex vivo* expanded human peripheral blood Nature Killer (PBNK) cells from healthy donors exhibited *in vitro* and *in vivo* anti-tumor activities against patient derived GBM cells (Lee, 2015). It was also reported that both CD133- medulloblastoma cells and CD133+ cancer stem cells were susceptible to NK cell-mediated killing *in vitro* (Castriconi, 2007). Ishikawa et al (2004) reported Phase I data for an intracranial and intravenous autologous NK cell injection that showed tumor regression in a small number of patients. Several ongoing clinical trials (ClinicalTrials.gov: NCT03360708, NCT01588769, NCT00331526, NCT03383978, NCT00003067) are investigating the potential of adoptive NK cell immunotherapy for GBM.

CYNK-001 is an allogeneic, off-the-shelf cell therapy enriched for CD56+/CD3- NK cells expanded from placental CD34+ cells. CYNK-001 exhibits *in vitro* cytotoxicity against GBM lines and secretes cytolytic cytokines during co-culture with cancer cells. Here, we have demonstrated that CYNK-001 administered via intracranial route exhibited *in vivo* anti-tumor activity in a U-87MG orthotopic mouse model. Furthermore, a single and repeat dose toxicity study showed that CYNK-001 with intracranial administration appears safe and well tolerated in NSG mice. Taken together, our data support the application of CYNK-001 cells for the development of an allogeneic adoptive immunotherapeutic for patients with GBM.

METHODS

In vivo efficacy of CYNK-001 via intracranial route of administration in U-87 MG orthotopic mouse model: Luciferase-expressing U-87 MG cells (1×10^4) were stereotactically injected into the cranium of NSG mice on Day 0. CYNK-001 at the dose of 0.5×10^6 cells/animal was administered via intracranial route on Day 14 and Day 25. Live bioluminescence imaging (BLI) was taken twice per week until study termination. In addition, clinical symptoms were closely monitored, and body weight was measured twice per week. The data were analyzed by GraphPad Prism.

In vivo efficacy of CYNK-001 via various routes of administration in U-87 MG orthotopic mouse model: Luciferase-expressing U-87 MG cells (1×10^4) were stereotactically injected into the cranium of NSG mice on Day 0. CYNK-001 at the dose of 0.5×10^6 cells/animal was administered via intracranial, intravenous or intracerebroventricular route on Day 7. Live bioluminescence imaging (BLI) was taken twice per week until study termination. In addition, clinical symptoms were closely monitored, and body weight was measured twice per week. The data were analyzed by GraphPad Prism.

In vivo safety and biodistribution of CYNK-001 with single or repeat intracranial administrations: Ninety-one female NSG mice were assigned to four groups to determine the tolerability, potential acute toxicity and biodistribution of CYNK-001. CYNK-001 or the vehicle was intracranially administered into striatum in a total volume of $5.0 \mu\text{L}$ using stereotaxic instrument on Day 0 for single dose or Days 0, 7 and 14 for repeat dose. For safety assessment, morbidity & mortality, clinical observations, body weight, gross pathology and histopathology were included. For biodistribution assessment, immunohistochemistry (IHC) detection of CYNK-001 cell markers and quantitative polymerase chain reaction (qPCR) for the human telomerase reverse transcriptase (hTERT) gene to detect the presence of human cells in various organs were performed. The data were analyzed by GraphPad Prism.

RESULTS

In Vivo Anti-tumor Activity of Intracranial Administered CYNK-001 in U-87 MG Orthotopic Mouse Model

In vivo anti-tumor activity of CYNK-001 via intracranial administration was first evaluated in the U-87 MG orthotopic GBM mouse model. As shown in Figure 1A, luciferase-expressing U-87 MG cells (1×10^4) were stereotactically injected into the cranium of NSG mice on Day 0. CYNK-001 at the dose of 0.5×10^6 cells/animal was administered via intracranial route on Day 14 and Day 25. No abnormal clinical symptoms were observed in the animals treated with CYNK-001. BLI signal showed that CYNK-001 significantly reduced tumor burden from Day 27 to Day 34 compared to the PBS control ($P < 0.05$) (Figure 1B).

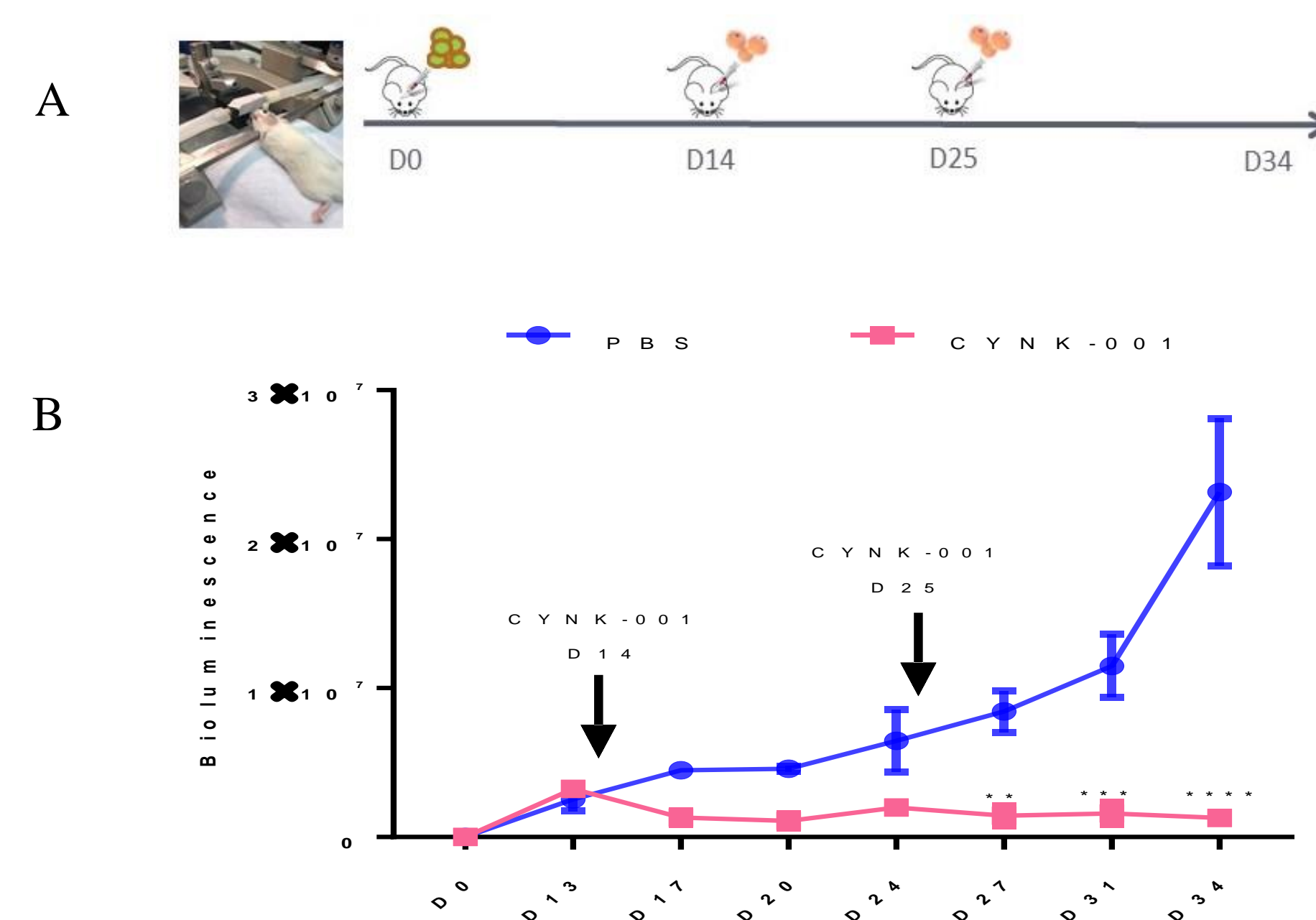


Figure 1. *In vivo* anti-tumor activity of CYNK-001 cells in U-87 MG orthotopic NSG mouse model. A) Study schema: U-87MG cells were intracranially injected on Day 0. PBS or CYNK-001 cells were intracranially injected on Days 14 and 25; B) BLI imaging. The data are presented as the mean \pm SEM; ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

In Vivo Anti-tumor Activity of CYNK-001 via Various Routes of Administration in U-87 MG Orthotopic Mouse Model

Anti-tumor activity of CYNK-001 via intracerebroventricular (ICV) or intravenous (IV) administration was further evaluated compared to intracranial (IC) administration in the U-87 MG orthotopic GBM mouse model. On Day 0, 1×10^4 luciferase-expressing U87 cells were stereotactically injected into the cranium of NSG mice. CYNK-001 cells were administered on Day 7 at the dose of 0.5×10^6 , 1×10^6 , and 10×10^6 for IC, ICV and IV routes, respectively. As shown in Figure 2, treatment with CYNK-001 cells delivered via the IC route resulted in lower average tumor burden in the treated mice on Day 26 after the tumor implantation as compared to the vehicle control. No differences in tumor growth were observed between CYNK-001 and corresponding vehicle-treated mice, when treatment was delivered via ICV or IV route of administration.

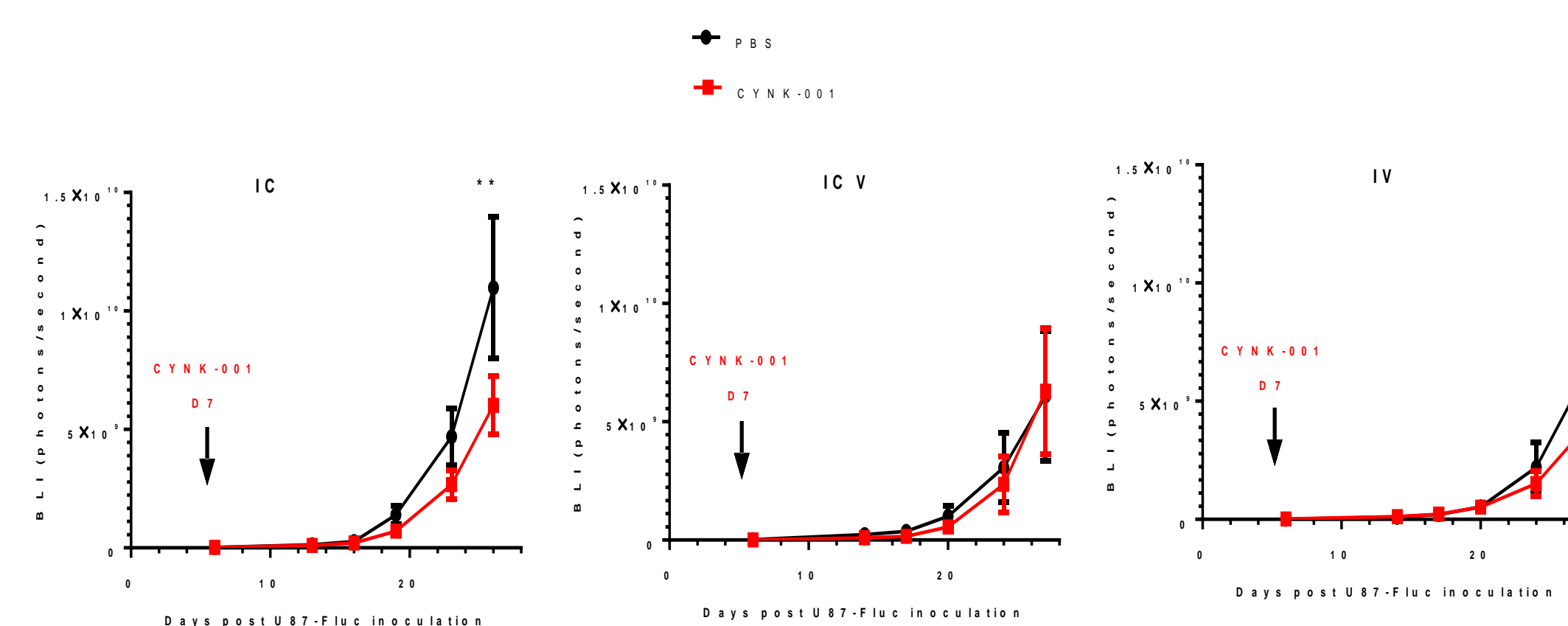


Figure 2. Anti-tumor activity of CYNK-001 with various routes of administration in orthotopic xenograft model. U-87MG cells were IC injected on Day 0. PBS or CYNK-001 cells were injected either IC, ICV, or IV. The data are presented as the mean \pm SEM; ** $P < 0.01$.

In Vivo Safety and Biodistribution of Intracranial Administered CYNK-001 in NSG Mice

Morbidity and Mortality

No animal was found at moribund condition and no unscheduled deaths occurred during the study.

Clinical symptoms

Mice were monitored for clinical signs of distress continuously during the first hour after injection, then every hour during the first four hours post injection, at twenty-four hours, and twice a week until termination. No adverse clinical symptoms were observed in animals that received a single or repeat IC injection of 0.5×10^6 CYNK-001 cells.

Gross pathology

No abnormal findings were observed during necropsy in CYNK-001 treated animals. No significant difference in organ weight was observed between CYNK-001 group and the vehicle group.

Histopathology

Histopathological evaluation was conducted on the fixed brain and spinal cord tissues from mice that received vehicle or CYNK-001. For single dose groups, histopathology was evaluated on Day 15. For repeat dose groups, histopathology was evaluated on Days 15 and 28. No treatment-related changes were seen in either the brain or spinal cord.

Biodistribution and persistence

qPCR analysis showed that human DNA was only detected in the brain, but not in the spinal cord, peripheral blood, bone marrow, lung, liver, spleen, heart and kidney. The persistence of CYNK-001 in the brain is less than 7 days post IC administration. These findings were corroborated by IHC analysis. As shown in Figure 3, human Ku80+ cells as well as Ku80+/Ki67+ cells were only detected in the brain of CYNK-001 treated animals.

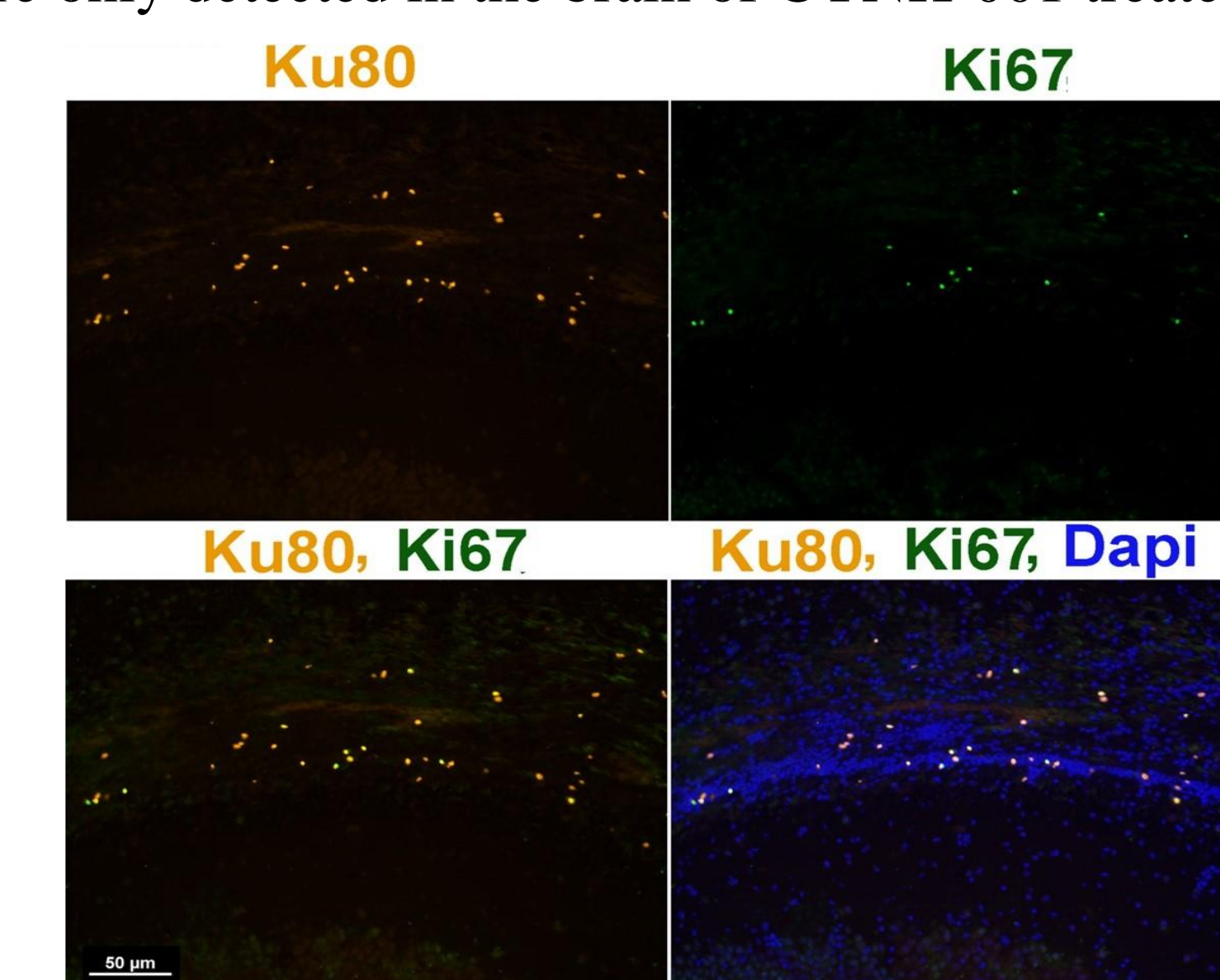


Figure 3. Immunohistochemical staining of human K80 and proliferation maker, Ki67, in the brain of CYNK-001 treated mouse on Day 1

SUMMARY & DISCUSSION

Our data demonstrated *in vivo* anti-tumor activity of CYNK-001 via IC route of administration in U-87 MG orthotopic mouse model. Furthermore, CYNK-001 with IC administration appears safe and well tolerated in NSG mice. The lack of efficacy via IV or ICV administration may be attributed to limited distribution to the brain through intact blood brain barrier (BBB) in the mouse model. However, the integrity of the BBB is disrupted in certain regions associated with tumor in GBM patients (Sarkaria, 2018), so NK cells administered via IV or ICV may penetrate through disrupted BBB and home to tumor. Taken together, our data support further development of adoptive transfer of CYNK-001 in patients with GBM.

REFERENCE

1. Castriconi R, et al. *Eur J Immunol.* 2007;37: 3190-3196.
2. Gittleman H et al. *Neuro Oncol.* 2018;20:6-16.
3. Ishikawa E et al. *Anticancer Res.* 2004;24(3b):1861-71.
4. Kamiya-Matsuoka C et al. *CNS Oncol.* 2015;4(2):91-104.
5. Lee et al. *BMC Cancer* 2015;15:1011
6. Sarkaria et al. *Neuro Oncol.* 2018; 20(2):184-191