As an alternative to bicistronic expression of the IL12 subunits, functional single chain IL12 fusion proteins have been produced by joining the p40 and p35 subunits with (Gly4Ser)3 or Gly6Ser linkers. Lieschke *et al*., (1997), Nature Biotechnology 15, 35-40; Lode *et al*., (1998), PNAS 95, 2475-2480. (These forms of p40-linker-p35 or p35-linker-p40 IL12 configurations may be referred to herein as "single chain IL12 (scIL12)").

Human IL12 p70 (i.e., dimer of p35 and p40) has a reported in vivo half-life of 5-19 hours which, when administered as a therapeutic compound, can result in significant systemic toxicity. See e.g., Car *et al*. "The Toxicology of Interleukin-12: A Review" *Toxicologic Path.* 27:1, 58-63 (1999); Robertson *et al*. "Immunological Effects of Interleukin 12 Administered by Bolus Intravenous Injection to Patients with Cancer" *Clin. Cancer Res.* 5:9-16 (1999); Atkins *et al*. "Phase I Evaluation of Intravenous Recombinant Human Interleukin 12 in Patients with Advance Malignancies" *Clin. Cancer Res.* 3:409-417 (1997). Preclinical studies in murine tumor treatment models demonstrate powerful antitumor effects following the systemic administration of IL12. In humans, however, attempts to systemically administer recombinant IL12 resulted in significant toxicities including patient deaths and limited efficacy. Thus, there remains a need in the art for improved therapeutic control of *in vivo* delivered forms of IL12.

SUMMARY

In one aspect, provided herein is a modified p40 domain comprising one or more amino acid substitutions at one or more positions of E45, D62 and D161 relative to the wild-type human mature IL12 p40 domain sequence set forth in SEQ ID NO: 10, wherein the one or more amino acid substitutions comprise one or more of D62H, D62I, D161R and D161S.

In another aspect, provided herein is a modified p40 domain comprising one or more amino acid substitutions relative to the wild-type human mature IL12 p40 domain sequence set forth in SEQ ID NO: 10, wherein the one or more amino acid substitutions comprise W15H, W15K, W15R, D18G, K58H, K58W, E59D, E59G, E59R, F60D, F60E, F60K, F60R, F60V, D62H, D62I, K84E, K84I, K84L, K84V, K84W, K84Y, E86L, E86R, E86S, E86W, D93E, D93H, D93R, D93W, D161R, D161S, K197D, K197E, K197Q, K197T, or K197W, or a combination thereof.

In another aspect, provided herein is a IL12 fusion protein comprising an IL12 polypeptide comprising a modified p40 domain described herein, and a p35 domain.

In another aspect, provided herein is a modified p40 domain described herein, a masked IL12 HetFc fusion protein described herein, or an unmasked IL12 HetFc fusion protein described herein, for use in therapy.

In another aspect, provided herein is the use of a modified p40 domain described herein, a masked IL12 HetFc fusion protein described herein, or an unmasked IL12 HetFc fusion protein described herein, in the preparation of a medicament for the treatment of cancer

One aspect of the present disclosure provides cytokine fusion proteins containing modified p40 domains and, in particular, provides IL12 and IL23 fusion proteins, also referred to herein as IL12 HetFc fusion proteins. The IL12 fusion proteins described herein can comprise an IL12 polypeptide, an Fc domain comprising a first Fc polypeptide and a second Fc polypeptide; and optionally a masking moiety (MM) that reduces, inhibits or blocks IL12 activity.

One aspect of the present disclosure describes a modified p40 domain, the modified p40 domain comprising one or more amino acid substitutions relative to the wild-type human mature IL12 p40 domain sequence set forth in SEQ ID NO: 10, wherein the one or more amino acid substitutions are located at one or more positions of E45, D62 and D161, and wherein the numbering of the amino acid residues is based on the amino acid sequence set forth in SEQ ID NO: 10.