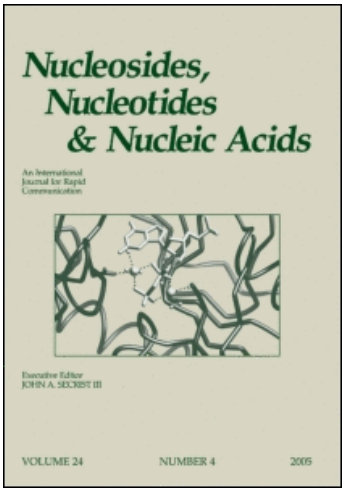

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Immune Cell Involvement in *Anti-c-myc* DNA Prevention of Tumor Formation in a Mouse Model of Burkitt'S Lymphoma

G. D. Gray[a]; R. Townsend[b]; H. Hayasaka[a]; R. Korngold[b]; E. Wickstrom[a]
[a] Department of Biochemistry and Molecular Pharmacology, Thomas Jefferson University, Philadelphia, Pennsylvania [b] Department of Microbiology and Immunology, Kimmel Cancer Center Thomas Jefferson University, Philadelphia, Pennsylvania

To cite this Article Gray, G. D. , Townsend, R. , Hayasaka, H. , Korngold, R. and Wickstrom, E.(1997) 'Immune Cell Involvement in *Anti-c-myc* DNA Prevention of Tumor Formation in a Mouse Model of Burkitt'S Lymphoma', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1727 — 1730
To link to this Article: DOI: 10.1080/07328319708006264
URL: http://dx.doi.org/10.1080/07328319708006264

NUCLEOSIDES & NUCLEOTIDES, 16(7–9), 1727–1730 (1997)

IMMUNE CELL INVOLVEMENT IN ANTI-C-*MYC* DNA PREVENTION OF TUMOR FORMATION IN A MOUSE MODEL OF BURKITT'S LYMPHOMA

Gray, G. D.[‡], Townsend, R.[†], Hayasaka, H.[‡], Korngold, R.[†], and Wickstrom, E.[‡*]
Departments of [‡]Biochemistry and Molecular Pharmacology, and
[†]Microbiology and Immunology, Kimmel Cancer Center
Thomas Jefferson University, Philadelphia, Pennsylvania 19107

ABSTRACT

Prophylactic therapy with 5'-dCACGTTGAGGGGCAT phosphorothioate, (2.5 nmol/hr) strongly inhibited tumorigenesis in Eμ-*myc* transgenic mice, and ablated spleen cell MYC antigen. However, the anti-c-*myc* DNA also stimulated proliferation of spleen cells *in vitro*, though much less so than a positive CG motif control, 5'-dGCATGACGTTGAGCT.

Transgenic mice bearing a murine immunoglobulin enhancer/c-*myc* fusion transgene (Eμ-*myc*) provide a useful model for Burkitt's lymphoma.[1] In recent work,[2] Eμ-*myc* mice treated by micro-osmotic pumps from 3-9 weeks after birth with saline vehicle or scrambled DNA phosphorothioate (2.5 nmol/hr, about 1 mg/kg/hr) displayed palpable tumors by 8-9 weeks of age, but 75% of Eμ-*myc* mice treated with anti-c-*myc* DNA phosphorothioate (2.5 nmol/hr) were still free of tumors at the age of 26 weeks. Anti-c-*myc* DNA therapy also ablated MYC antigen in the spleens of tumor-bearing mice.

DNA phosphorothioates delivered by this regimen reached a steady-state serum concentration of 0.1 μM, measured by fluorescence induced by addition of OliGreen™ (Molecular Probes, Eugene OR).[3] The anti-c-*myc* sequence, 5'-dCACGTTGAGGGGCAT, is potentially capable of forming a four-strand tetraplex via the G_4 tracts, and does so at 4° and 23°.[4] However, this tetraplex is not stable at 37° in physiological salts, even in the presence of serum, and the preformed tetraplex dissociates rapidly at 37°.[4]

In subsequent experiments at lower dose rates, 1.25 nmol/hr, 0.625 nmol/hr, and 0.313 nmol/hr, proportionately shorter tumor onset times were observed, with no significant protection at the lowest dose rate, or by the scrambled control (Fig. 1).

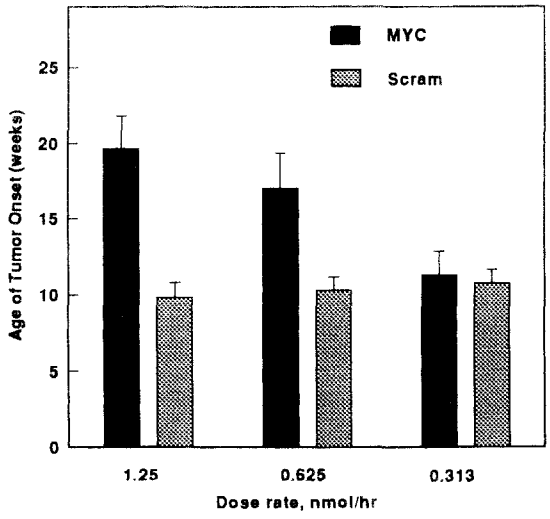

FIG. 1 Age of tumor onset in Eμ-*myc* transgenic mice treated from 3-9 weeks of age with micro-osmotic pumps containing anti-c-*myc* or scrambled oligonucleotide at 1.25 nmol/hr, 0.625 nmol/hr, or 0.313 nmol/hr. Data represent mean ± SE of 8-11 animals.

Longterm tumor resistance after anti-c-*myc* DNA therapy suggests the induction of a host immune response. The anti-c-*myc* sequence, 5'-dCACGTTGAGGGGCAT, shares some homology with the PuPuCGPyPy motif which activates B and T cells *in vitro*.[5,6] Therefore stimulation of non-transgenic spleen cell proliferation was compared *in vitro* among the anti-c-*myc* sequence, a strong immunostimulatory CG sequence, 5'-dGCATGACGTTGAGCT,[5] and the scrambled control (Fig. 2). Both the anti-c-*myc* and CG control sequences increased cell numbers over 8-fold in 60 hr. at 3 and 9 μM. The CG sequence was also a strong stimulator at 0.1 or 0.3 μM, but not

the anti-c-*myc* sequence. Similar results were found for Eμ-*myc* transgenic mouse spleen cells (not shown). Since we found a steady-state oligonucleotide serum concentration of 0.1 μM in mice treated subcutaneously from micropumps at 2.5 nmol/hr,[3] the results in Fig. 2 suggest that immune cell stimulation by anti-c-*myc* DNA may not be a powerful effect *in vivo* under this regimen, which greatly reduced tumor formation in Eμ-*myc* transgenic mice.[2]

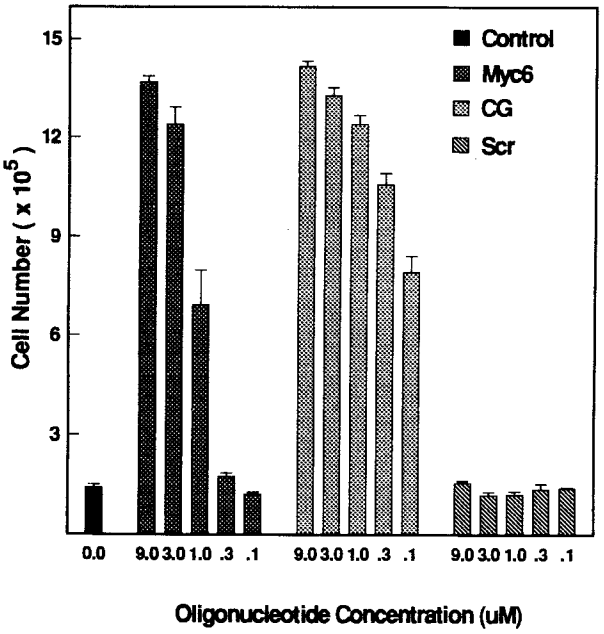

FIG. 2 Spleen cell proliferation following treatment *in vitro* with anti-c-*myc* (Myc6), CG, or scrambled DNA, vs. untreated cells (Control). Spleen cells were obtained from a male (C57BL6xSJL6) F_1 mouse and plated in RPMI 1640 with 10% FBS in a 96-well plate at 2 x 10^5 cells per well. Oligonucleotides were added to the cell medium at varying concentrations, and the cells were incubated for 60 hr at 37°, after which cell numbers were evaluated using AlamarBlue™ metabolic dye. Data represent means ± SE of four replicates.

Examination of length and sequence dependence of spleen cell stimulation at 3 μM oligonucleotide (Fig. 3) revealed that 5' and 3' decamer fragments of the anti-c-*myc* sequence were not stimulatory, nor was the 5' hexamer. A pentadecamer with two central mismatches showed full stimulation, while pentadecamers with 5' or 3' scrambled hexamers, or a 3'-5' reversed sequence, induced 2-3-fold stimulation.

Stimulation of B cells vs. T cells was compared by separating B cells and T cells from spleen cells collected from an untreated Eμ-*myc* transgenic mouse presenting numerous lymphomas and pronounced splenomegaly. All three cell populations demonstrated a strong

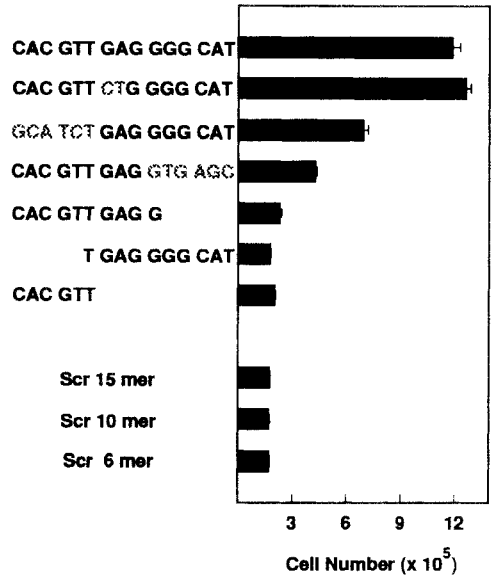

FIG. 3 Spleen cell proliferation *in vitro* as a function of base mutations or deletions in the anti-c-*myc* sequence, shown at the top left. The sequences below contain base substitutions (gray letters) or deletions. Spleen cells were treated with 3 μM oligonucleotide, and analyzed as in Fig. 2. Data represent means ± SE of four replicates.

proliferative response to both anti-c-*myc* DNA, and to the CG positive control, at 9 μM oligonucleotide (not shown).

With respect to the mechanism(s) of action of anti-c-*myc* DNA in limiting tumorigenesis, the key questions to be addressed concern sequence and dose dependence. Longterm tumor prevention studies in Eμ-*myc* mice are necessary to determine the extent of immune cell stimulation *vs.* antisense effects by the anti-c-*myc* sequence and control sequences at the oligonucleotide concentrations experienced *in vivo*.

We thank Dr. Michael Kligshteyn for oligonucleotide synthesis, and Irina Feldman for assistance with the animal studies. This work was supported by grants from NIH (CA42960 to E. W., CA60630 to R. K.) and Snow Brand Milk Products (to E. W.).

REFERENCES

1. Wickstrom, E.; Bacon, T. A.; Wickstrom, E. L. *Cancer Res.* **1992**, *52*, 6741-6745.
2. Huang, Y.; Snyder, R.; Kligshteyn, M.; Wickstrom, E. *Molec. Med.* **1995**, *1*, 647-658.
3. Gray, G. D.; Wickstrom, E. submitted for publication, **1996.**
4. Basu, S.; Wickstrom, E. American Society for Biochemistry and Molecular Biology Annual Meeting, New Orleans, Louisiana, **1996**, 2848.
5. Krieg, A. M.; Yi, A.-Y.; Matson, S.; Waldschmidt, T. J.; Bishop, G. A.; Teasdale, R.; Koretzky, G. A.; Klinman, D. M. *Nature* **1995**, *374*, 546-549.
6. Klinman, D. M.; Yi, A.-Y.; Beaucage, S.L.; Conover, J.; Krieg, A. M. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2879-2883.