

Neuroblastoma: A Malignancy Due to Cell Differentiation Block

Andrew Tee, Pei Y. Liu, Glenn M. Marshall and Tao Liu
*Children's Cancer Institute Australia for Medical Research
Australia*

1. Introduction

Neuroblastoma originates from precursor neuroblasts of the sympathetic nervous system. Unlike most other cancer types, neuroblastoma is characterized by a unique capacity for spontaneous complete regression, at least partly through neuronal differentiation, in a proportion of patients, and is therefore regarded as a cancer due to cell differentiation block. The first demonstration of *in vitro* differentiation of human neuroblastoma cells was published 30 years ago, when human SK-N-SH and SH-SY5Y neuroblastoma cells were shown to differentiate morphologically (neurite outgrowth) and biochemically in response to 12-O-tetradecanoyl-phorbol-13-acetate (TPA) treatment (Påhlman et al., 1981). The differentiated cells showed an increased expression of noradrenaline, adrenaline and neuron-specific enolase (NSE), differentiation markers which are employed for the diagnosis of neuroblastoma in patients. In 1982, retinoic acid (RA) was shown to induce concentration-dependent morphologic differentiation and growth inhibition in the LA-N-1 human neuroblastoma cell line (Sidell, 1982). The RA-induced morphologic differentiation and growth inhibition persisted despite removal of the drug. These observations demonstrate that RA promotes the differentiation of LA-N-1 neuroblastoma cells and results in a reduced expression of the malignant phenotype, and suggest that patients with advanced neuroblastoma may be successfully treated by RA to induce their tumour cells to differentiate and to undergo growth inhibition. In the last three decades, a number of factors, such as up-regulation of the expression of retinoic acid receptors, have been identified as crucial for the induction or blockage of neuroblastoma cell differentiation. Importantly, naturally occurring and synthetic retinoids have shown great promise in the clinic when used as differentiation agents in neuroblastoma patients with minimal residual disease, while the mechanism of retinoid anticancer signalling in neuroblastoma is still not fully understood (Liu et al., 2005; Reynolds et al., 2003).

2. Neurotrophin and neurotrophin receptor-induced neuroblastoma cell differentiation

Among the best characterized factors involved in pre-cancerous neuroblast and neuroblastoma cell differentiation are the tyrosine kinase receptor (TRK), its ligands nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-5). Neuroblastoma cell lines generally lack functional neurotrophin receptors of the TRK gene family and therefore do not differentiate when stimulated with

neurotrophins including NGF, BDNF, NTF3 or NTF5, and absence of TRKA and TRKC gene expression in neuroblastoma tissues correlates with poor prognosis in neuroblastoma patients (Kogner et al., 1993; Suzuki et al., 1993; Yamashiro et al., 1996). When transfected with either TRKA or TRKC, neuroblastoma cell lines can differentiate into sympathetic neurons in response to treatment with their cognate ligands NGF and NT-3 (Edsjo et al., 2001; Lavenius et al., 1995). Moreover, treatment with growth inhibition agents such as aphidicolin and all-trans RA (atRA) results in NGF responsiveness through induction of TRKA gene expression, demonstrating that TRK-negative cells retain a capacity to respond to TRK-mediated cell differentiation (Hishiki et al., 1998; Poluha, Poluha, and Ross, 1995). Taken together, these data show that many neuroblastoma cell lines have the capacity to respond to physiological differentiation stimuli, although they have lost this capacity with regard to stimulation with NGF or with other neurotrophins unless the neuroblastoma cells are triggered to express the cognate receptors [reviewed in (Edsjö, Holmquist, and Pählman, 2007)].

While TRKA- and TRKC-mediated signalling decreases neuroblastoma cell proliferation and aggressiveness, BDNF/TRKB signalling corresponds with poor outcome in neuroblastoma patients [reviewed in (Edsjö, Holmquist, and Pählman, 2007)]. BDNF treatment of neuroblastoma cells transfected with a TRKB over-expressing construct does not induce differentiation (Ho et al., 2011), and BDNF stimulation of neuroblastoma cell lines expressing TRKB as a result of RA treatment does not affect proliferation, but increases survival and invasiveness (Matsumoto et al., 1995). In addition to these effects of TRKA and TRKB signalling, recent data also suggest a difference with respect to therapy resistance, invasiveness, angiogenesis, and possibly also genomic stability (Schramm et al., 2005).

atRA treatment of neuroblastoma cells can also induce the expression of the proto-oncogene RET, which codes for a tyrosine kinase growth factor receptor specifically binding glial cell line-derived neurotrophic factor (GDNF) (Airaksinen and Saarma, 2002). GDNF is required for the proper development of enteric and parasympathetic neuroblasts (Airaksinen and Saarma, 2002), and atRA-treated neuroblastoma cells differentiate further in response to GDNF (Hishiki et al., 1998). Additionally, activation of RET by GDNF leads to differentiation of neuroblasts and neuroblastoma cells into ganglia cells (D'Alessio et al., 1995), and GDNF synergizes with ciliary neurotrophic factor (CNTF) to enhance TRKA receptor expression, thereby strengthening NGF-mediated cell differentiation signal (Peterson and Bogenmann, 2004).

3. Fyn and Fyn kinase induce neuroblastoma cell differentiation

Other factors involved in neuroblastoma cell differentiation include the Fyn nonreceptor kinase. Expression of active Fyn induces differentiation and growth arrest in neuroblastoma cells (Berwanger et al., 2002). High expression of Fyn and high Fyn kinase activity are restricted to low-stage human neuroblastoma tissues, and expression of Fyn predicts long-term survival independently of MYCN gene amplification (Berwanger et al., 2002).

4. N-Myc blocks neuroblastoma cell differentiation

The most powerful endogenous blocker of neuroblastoma cell differentiation is the MYCN oncogene. N-Myc is well-known to repress neuroblastoma cell differentiation partly through repressing TRKA gene expression. A recent study demonstrates that N-Myc blocks TRKA gene expression by recruiting histone deacetylase 1 (HDAC1) to TRKA gene promoter and repressing its transcription (Iraci et al., 2011). Additionally, N-Myc represses the expression of growth arrest specific1 (GAS1), leading to increased RET tyrosine 1062 phosphorylation and decreased GDNF signaling (Lopez-Ramirez et al., 2008).

5. Histone deacetylase 1 and tissue transglutaminase in neuroblastoma cell differentiation

The transamidation activity of tissue transglutaminase (TG2) has been shown to be essential for the neuroblastoma and leukemia cell differentiation response to retinoid therapy (Balajthy et al., 2006; Tucholski, Lesort, and Johnson, 2001), and TG2 overexpression alone induces neuronal differentiation in neuroblastoma cells (Tucholski, Lesort, and Johnson, 2001). We have recently shown that repression of TG2 gene expression and reduction in transamidation activity are essential for N-Myc-induced neuroblastoma cell differentiation block (Liu et al., 2007). Importantly, dual step cross-linking chromatin immunoprecipitation and protein coimmunoprecipitation assays show that N-Myc acts as a transrepressor by recruiting HDAC1 protein to an Sp1-binding site in the TG2 gene core promoter in a manner distinct from its action as a transactivator at E-Box binding sites (Liu et al., 2007). Histone deacetylase inhibitor treatment blocks the N-Myc-mediated HDAC1 recruitment and TG2 repression *in vitro*. In neuroblastoma-bearing N-Myc transgenic mice, histone deacetylase inhibitor treatment induces TG2 expression and demonstrates marked antitumor activity *in vivo*. Taken together, these data indicate the critical roles of HDAC1 and TG2 in N-Myc-induced oncogenesis and have significant implications for the use of histone deacetylase inhibitor therapy in N-Myc-driven neuroblastoma.

6. Opposing effects of two tissue transglutaminase protein isoforms in neuroblastoma cell differentiation

Uniquely, two isoforms of TG2 mRNA and protein, a short form (TG2-S) and a full length form (TG2-L), have been characterized (Begg et al., 2006). We have shown that TG2-L and TG2-S exert opposing effects on cell differentiation (Tee et al., 2010). Repression of TG2-L with small interfering RNA, which does not affect TG2-S expression, induces dramatic neuronal differentiation in neuroblastoma cells. In contrast, overexpression of TG2-S or a GTP-binding-deficient mutant of TG2-L (R580A), both of which lack the GTP-binding Arg-580 residue, induces neuroblastoma cell differentiation, which is blocked by an inhibitor of transamidase activity. Whereas N-Myc represses and retinoid activates both TG2 isoforms, repression of TG2-L, but not simultaneous repression of TG2-L and TG2-S, enhances neuroblastoma cell differentiation due to N-Myc small interfering RNA or retinoid. Moreover, suppression of vasoactive intestinal peptide (VIP) expression alone induces neuroblastoma cell differentiation, and VIP can be up-regulated by TG2-L, but not TG2-S. Taken together, these data indicate that TG2-L and TG2-S exert opposite effects on cell differentiation due to differences in GTP binding and modulation of VIP gene transcription (Tee et al., 2010).

7. Retinoid differentiation therapy

Conventional therapy of neuroblastoma patients now includes the differentiation agent retinoid. Unlike chemo-radiotherapy, retinoid differentiation therapy shows minimal side effects on normal cells, because normal non-malignant cells are already differentiated. The effects of retinoids are mediated by two classes of non-steroid nuclear hormone receptors, the retinoic acid (RAR α , β , γ) and the retinoic X (RXR α , β , γ) receptors (Reynolds et al., 2003). The naturally occurring all-trans-retinoic acid (atRA), 9-cis-retinoic acid and the synthetic 13-cis-retinoic acid (13-cis-RA) are examples of retinoids studied in neuroblastoma. It has long been established that atRA stimulation of neuroblastoma cells results in growth inhibition and neuronal differentiation as indicated by neurite outgrowth, increased NSE

activity and accumulation of norepinephrine (Pahlman et al., 1984; Sidell, 1982). Even though atRA induces neuronal differentiation, the outcome differs depending on the neuroblastoma cell line studied. While some cell lines develop a sympathetic noradrenergic phenotype, a cholinergic switch has been suggested in other cell lines (Handler et al., 2000; Hill and Robertson, 1997). Mechanism studies have revealed that the down-regulation of the proto-oncogenes N-Myc, MYB and HRAS precedes the morphological differentiation, with changes in the expression of other proto-oncogenes following thereafter (Thiele, Deutsch, and Israel, 1988; Thiele, Reynolds, and Israel, 1985). As discussed in the previous sections, atRA treatment can also result in neuroblastoma cell differentiation by up-regulating the expression of the neurotrophin receptors TRKA, TRKB and RET, leading to responsiveness to their ligands NGF, BDNF and GDNF (Cerchia et al., 2006; Hishiki et al., 1998).

Phase I clinical trials show that higher and more sustained drug levels can be obtained with 13-cis-RA relative to atRA, and a phase III randomized clinical trial shows that high-dose, pulse therapy with 13-cis-RA given after completion of intensive chemoradiotherapy (with or without autologous bone marrow transplantation) significantly improved event-free survival in high-risk neuroblastoma patients (Reynolds et al., 2003) (Matthay et al., 1999). Moreover, *In vitro* as well as clinical data indicate that synthetic retinoids such as Fenretinide might be effective against neuroblastoma cells resistant to 13-cis-RA therapy. This effect seems to be independent of retinoid receptors, does not involve differentiation but is involved in the induction of programmed cell death (Reynolds et al., 2003).

8. Combination differentiation therapy with retinoids and histone deacetylase inhibitors

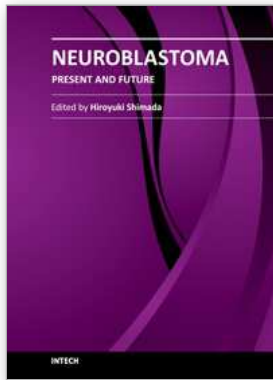
Although 13-cis-RA significantly improves event-free survival in high-risk neuroblastoma patients (Matthay et al., 1999), resistance to retinoid therapy eventually develops in more than 50% of neuroblastoma patients. Combination therapies with retinoids and other anti-cancer agents have therefore been studied extensively. Interestingly, during screening a small-molecule library for compounds enhancing histone deacetylase inhibitor-induced neuroblastoma cell differentiation, retinoids were found to be the top hit compounds (Hahn et al., 2008). Secondary assays confirmed greater neuroblastoma differentiation with the combination therapy of histone deacetylase inhibitors and retinoids. In a xenograft model of neuroblastoma, animals treated with the combination therapy with the histone deacetylase inhibitor SAHA and atRA had the longest survival. This work suggests that combination therapy with histone deacetylase inhibitors and retinoids could be a promising new strategy for differentiation therapy of children with neuroblastoma (Hahn et al., 2008).

9. References

- Airaksinen, M. S., and Saarma, M. (2002). The GDNF family: Signalling, biological functions and therapeutic value. *Nat Rev Neurosci* 3(5), 383-394.
- Balajthy, Z., Csomos, K., Vamosi, G., Szanto, A., Lanotte, M., and Fesus, L. (2006). Tissue-transglutaminase contributes to neutrophil granulocyte differentiation and functions. *Blood* 108(6), 2045-54.
- Begg, G. E., Carrington, L., Stokes, P. H., Matthews, J. M., Wouters, M. A., Husain, A., Lorand, L., Iismaa, S. E., and Graham, R. M. (2006). Mechanism of allosteric regulation of transglutaminase 2 by GTP. *Proc Natl Acad Sci U S A* 103(52), 19683-8.
- Berwanger, B., Hartmann, O., Bergmann, E., Bernard, S., Nielsen, D., Krause, M., Kartal, A., Flynn, D., Wiedemeyer, R., Schwab, M., Schafer, H., Christiansen, H., and Eilers, M.

- (2002). Loss of a FYN-regulated differentiation and growth arrest pathway in advanced stage neuroblastoma. *Cancer Cell* 2(5), 377-86.
- Cerchia, L., D'Alessio, A., Amabile, G., Duconge, F., Pestourie, C., Tavitian, B., Libri, D., and de Franciscis, V. (2006). An autocrine loop involving ret and glial cell-derived neurotrophic factor mediates retinoic acid-induced neuroblastoma cell differentiation. *Mol Cancer Res* 4(7), 481-8.
- D'Alessio, A., De Vita, G., Cali, G., Nitsch, L., Fusco, A., Vecchio, G., Santelli, G., Santoro, M., and de Franciscis, V. (1995). Expression of the RET oncogene induces differentiation of SK-N-BE neuroblastoma cells. *Cell Growth Differ* 6(11), 1387-94.
- Edsjo, A., Hallberg, B., Fagerstrom, S., Larsson, C., Axelson, H., and Pahlman, S. (2001). Differences in Early and Late Responses between Neurotrophin-stimulated trkA- and trkC-transfected SH-SY5Y Neuroblastoma Cells. *Cell Growth Differ* 12(1), 39-50.
- Edsjö, A., Holmquist, L., and Pählman, S. (2007). Neuroblastoma as an experimental model for neuronal differentiation and hypoxia-induced tumor cell dedifferentiation. *Semin Cancer Biol.* 17(3), 248-56.
- Hahn, C. K., Ross, K. N., Warrington, I. M., Mazitschek, R., Kanegai, C. M., Wright, R. D., Kung, A. L., Golub, T. R., and Stegmaier, K. (2008). Expression-based screening identifies the combination of histone deacetylase inhibitors and retinoids for neuroblastoma differentiation. *Proc Natl Acad Sci U S A* 105(28), 9751-6.
- Handler, A., Lobo, M. D., Alonso, F. J., Paino, C. L., and Mena, M. A. (2000). Functional implications of the noradrenergic-cholinergic switch induced by retinoic acid in NB69 neuroblastoma cells. *J Neurosci Res* 60(3), 311-20.
- Hill, D. P., and Robertson, K. A. (1997). Characterization of the cholinergic neuronal differentiation of the human neuroblastoma cell line LA-N-5 after treatment with retinoic acid. *Brain Res Dev Brain Res* 102(1), 53-67.
- Hishiki, T., Nimura, Y., Isogai, E., Kondo, K., Ichimiya, S., Nakamura, Y., Ozaki, T., Sakiyama, S., Hirose, M., Seki, N., Takahashi, H., Ohnuma, N., Tanabe, M., and Nakagawara, A. (1998). Glial cell line-derived neurotrophic factor/neurturin-induced differentiation and its enhancement by retinoic acid in primary human neuroblastomas expressing c-Ret, GFR alpha-1, and GFR alpha-2. *Cancer Res* 58(10), 2158-65.
- Ho, R., Minturn, J. E., Simpson, A. M., Iyer, R., Light, J. E., Evans, A. E., and Brodeur, G. M. (2011). The effect of P75 on Trk receptors in neuroblastomas. *Cancer Lett* 305(1):76-85.
- Iraci, N., Diolaiti, D., Papa, A., Porro, A., Valli, E., Gherardi, S., Herold, S., Eilers, M., Bernardoni, R., Della Valle, G., and Perini, G. (2011). A SP1/MIZ1/MYCN repression complex recruits HDAC1 at the TRKA and p75NTR promoters and affects neuroblastoma malignancy by inhibiting the cell response to NGF. *Cancer Res* 71(2), 404-12.
- Kogner, P., Barbany, G., Dominici, C., Castello, M. A., Raschella, G., and Persson, H. (1993). Coexpression of messenger RNA for TRK protooncogene and low affinity nerve growth factor receptor in neuroblastoma with favorable prognosis. *Cancer Res* 53(9), 2044-50.
- Lavenius, E., Gestblom, C., Johansson, I., N°anberg, E., and P°ahlman, S. (1995). Transfection of TRK-A into human neuroblastoma cells restores their ability to differentiate in response to nerve growth factor. *Cell Growth Differ* 6, 727-36.
- Liu, T., Bohlken, A., Kuljaca, S., Lee, M., Nguyen, T., Smith, S., Cheung, B., Norris, M. D., Haber, M., Holloway, A. J., Bowtell, D. D., and Marshall, G. M. (2005). The retinoid anticancer signal: mechanisms of target gene regulation. *Br J Cancer* 93(3), 310-8.

- Liu, T., Tee, A. E., Porro, A., Smith, S. A., Dwarthe, T., Liu, P. Y., Iraci, N., Sekyere, E., Haber, M., Norris, M. D., Diolaiti, D., Della Valle, G., Perini, G., and Marshall, G. M. (2007). Activation of tissue transglutaminase transcription by histone deacetylase inhibition as a therapeutic approach for Myc oncogenesis. *Proc Natl Acad Sci U S A* 104(47), 18682-7.
- Lopez-Ramirez, M. A., Dominguez-Monzon, G., Vergara, P., and Segovia, J. (2008). Gas1 reduces Ret tyrosine 1062 phosphorylation and alters GDNF-mediated intracellular signaling. *Int J Dev Neurosci* 26(5), 497-503.
- Matsumoto, K., Wada, R., Yamashiro, J., Kaplan, D., and Thiele, C. (1995). Expression of brain-derived neurotrophic factor and p145TrkB affects survival, differentiation, and invasiveness of human neuroblastoma cells. *Cancer Res* 55, 1798-806.
- Matthay, K. K., Villablanca, J. G., Seeger, R. C., Stram, D. O., Harris, R. E., Ramsay, N. K., Swift, P., Shimada, H., Black, C. T., Brodeur, G. M., Gerbing, R. B., and Reynolds, C. P. (1999). Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. *N Engl J Med* 341(16), 1165-73.
- Påhlman, S., Odelstad, L., Larsson, E., Grotte, G., and Nilsson, K. (1981). Phenotypic changes of human neuroblastoma cells in culture induced by 12-Otetradecanoyl-phorbol-13-acetate. *Int J Cancer* 28, 583-9.
- Pahlman, S., Ruusala, A. I., Abrahamsson, L., Mattsson, M. E., and Esscher, T. (1984). Retinoic acid-induced differentiation of cultured human neuroblastoma cells: a comparison with phorbol-ester-induced differentiation. *Cell Differ* 14(2), 135-44.
- Peterson, S., and Bogenmann, E. (2004). The RET and TRKA pathways collaborate to regulate neuroblastoma differentiation. *Oncogene* 23(1), 213-25.
- Poluha, W., Poluha, D. K., and Ross, A. H. (1995). TrkA neurogenic receptor regulates differentiation of neuroblastoma cells. *Oncogene* 10(1), 185-9.
- Reynolds, C. P., Matthay, K. K., Villablanca, J. G., and Maurer, B. J. (2003). Retinoid therapy of high-risk neuroblastoma. *Cancer Lett* 197(1-2), 185-92.
- Schramm, A., Schulte, J., Astrahantseff, K., Apostolov, O., Limpt, V., and Sieverts, H. (2005). Biological effects of TrkA and TrkB receptor signaling in neuroblastoma. *Cancer Lett* 228, 143-53.
- Sidell, N. (1982). Retinoic acid-induced growth inhibition and morphologic differentiation of human neuroblastoma cells in vitro. *J Natl Cancer Inst* 68(4), 589-96.
- Suzuki, T., Bogenmann, E., Shimada, H., Stram, D., and Seeger, R. C. (1993). Lack of high-affinity nerve growth factor receptors in aggressive neuroblastomas. *J Natl Cancer Inst* 85(5), 377-84.
- Tee, A. E., Marshall, G. M., Liu, P. Y., Xu, N., Haber, M., Norris, M. D., Iismaa, S. E., and Liu, T. (2010). Opposing effects of two tissue transglutaminase protein isoforms in neuroblastoma cell differentiation. *J Biol Chem* 285(6), 3561-7.
- Thiele, C. J., Deutsch, L. A., and Israel, M. A. (1988). The expression of multiple proto-oncogenes is differentially regulated during retinoic acid induced maturation of human neuroblastoma cell lines. *Oncogene* 3(3), 281-8.
- Thiele, C. J., Reynolds, C. P., and Israel, M. A. (1985). Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma. *Nature* 313(6001), 404-6.
- Tucholski, J., Lesort, M., and Johnson, G. V. (2001). Tissue transglutaminase is essential for neurite outgrowth in human neuroblastoma SH-SY5Y cells. *Neuroscience* 102(2), 481-91.
- Yamashiro, D. J., Nakagawara, A., Ikegaki, N., Liu, X. G., and Brodeur, G. M. (1996). Expression of TrkC in favorable human neuroblastomas. *Oncogene* 12(1), 37-41.



Neuroblastoma - Present and Future

Edited by Prof. Hiroyuki Shimada

ISBN 978-953-307-016-2

Hard cover, 366 pages

Publisher InTech

Published online 08, February, 2012

Published in print edition February, 2012

Neuroblastoma, once called "enigmatic", due to "unpredictable" clinical behaviors, is composed of biologically diverse tumors. Molecular/genomic properties unique to the individual tumors closely link to the clinical outcomes of patients. Establishing risk stratification models after analyzing biologic characteristics of each case has made a great success in patient management. However, the trend of improving survival rates in neuroblastoma over the last 30 years has started to level off, and currently available treatment modalities have almost reached to their maximized intensity. Furthermore, aggressive treatment causes significant long-term morbidities to the survivors. We really need to make the next step to the level of personalized medicine with more precise understanding of neuroblastoma biology. This book includes useful data and insights from the world's experts in this field. I believe this book can make an excellent contribution to all the investigators working hard and fighting for the children stricken by this disease.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Andrew Tee, Pei Y. Liu, Glenn M. Marshall and Tao Liu (2012). Neuroblastoma: A Malignancy Due to Cell Differentiation Block, Neuroblastoma - Present and Future, Prof. Hiroyuki Shimada (Ed.), ISBN: 978-953-307-016-2, InTech, Available from: <http://www.intechopen.com/books/neuroblastoma-present-and-future/neuroblastoma-a-malignancy-due-to-cell-differentiation-block>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.