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Supplementary Materials for

Bacteria-Induced Gap Junctions in Tumors Favor Antigen Cross-Presentation and Antitumor Immunity

Fabiana Saccheri, Chiara Pozzi, Francesca Avogadri, Sara Barozzi, Mario Faretta, Paola Fusi, Maria Rescigno*

*To whom correspondence should be addressed. E-mail: maria.rescigno@ifom-ieo-campus.it

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Other Supplementary Material for this manuscript includes the following:
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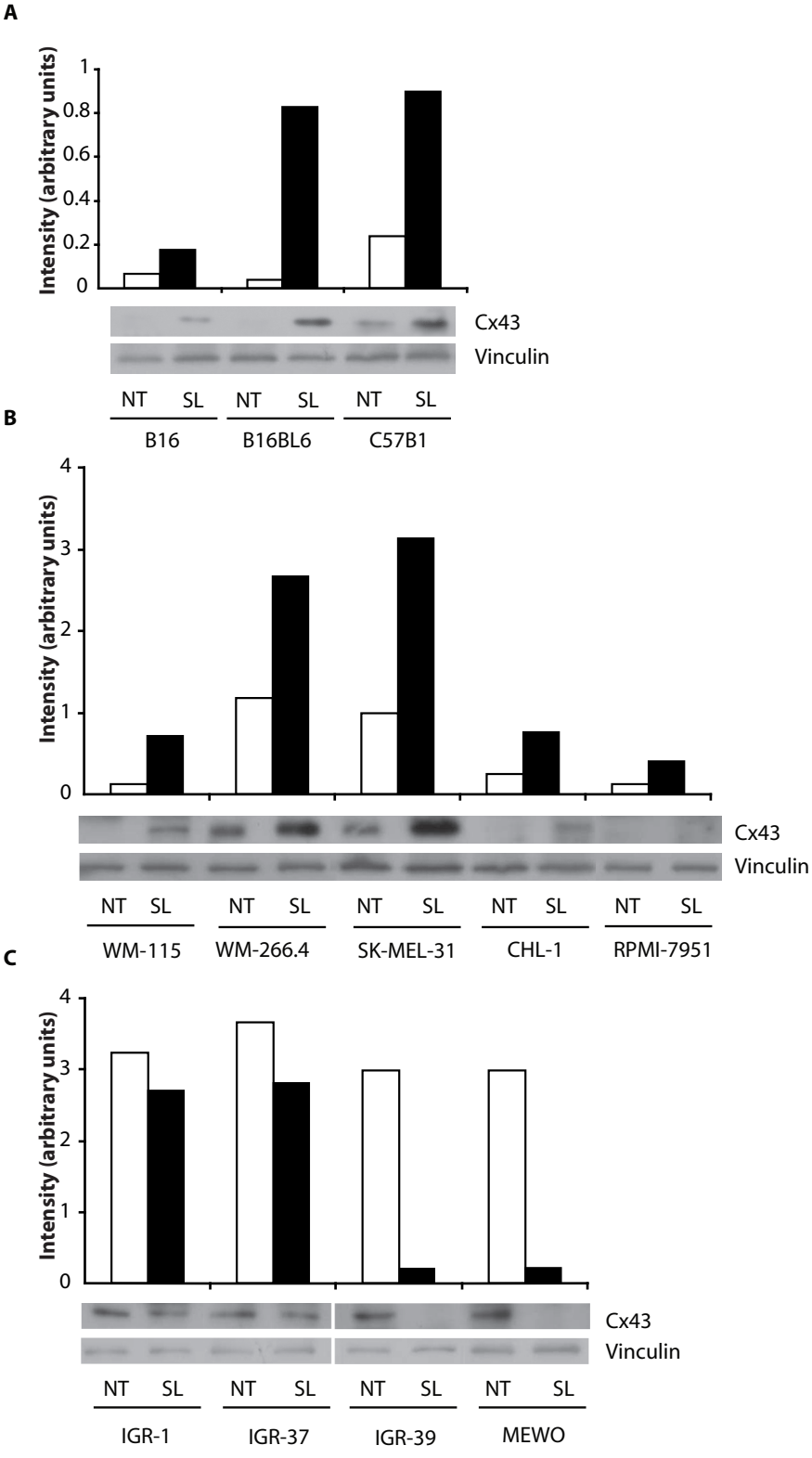
A
Intensity (arbitrary units)
1
0.8
0.6
0.4
0.2
0
Cx43
Vinculin
NT SL NT SL NT SL
B16 B16BL6 C57B1
B
Intensity (arbitrary units)
4
3
2
1
0
Cx43
Vinculin
NT SL NT SL NT SL NT SL NT SL
WM-115 WM-266.4 SK-MEL-31 CHL-1 RPMI-7951
C
Intensity (arbitrary units)
4
3
2
1
0
Cx43
Vinculin
NT SL NT SL NT SL NT SL
IGR-1 IGR-37 IGR-39 MEWO

Fig. S1. All of the murine and half of the human melanoma cell lines up-regulate Cx43 in response to *Salmonella*. Murine (**A**) and human (**B-C**) melanoma cell lines were incubated (SL) or not (NT) with *Salmonella* in medium without antibiotics for 2 h. 24 h later, cells were analyzed for Cx43 expression by Western blot. Vinculin was used as reference protein. Bars represent the quantification of the bands of cells treated (black bars) or not (white bars) with *Salmonella*. The intensity of the band is shown as arbitrary units.

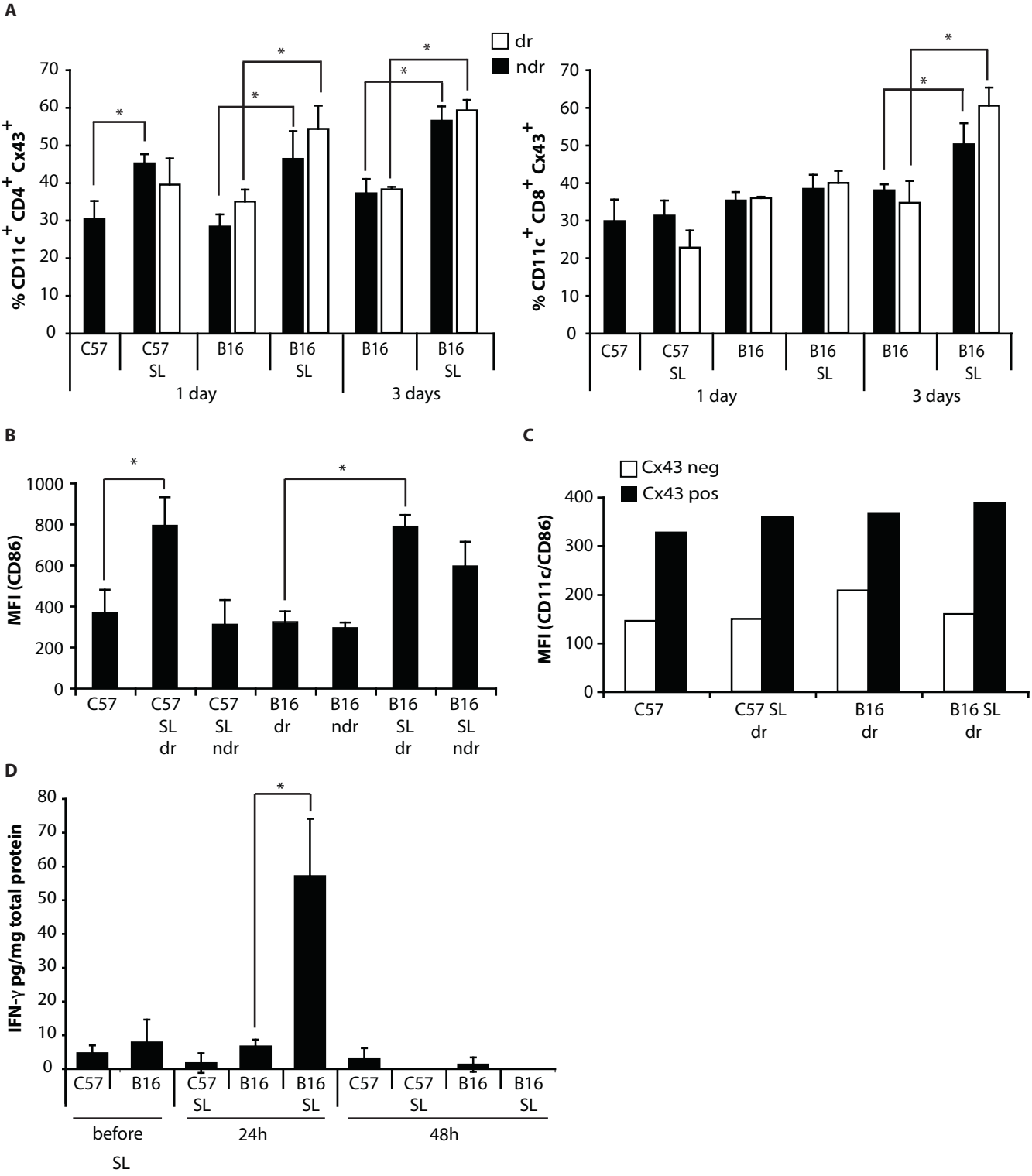
A
dr
ndr
% CD11c+ CD4+ Cx43+
% CD11c+ CD8+ Cx43+
C57
C57 SL
B16
B16 SL
1 day
3 days
B
MFI (CD86)
C57 SL dr
C57 SL ndr
B16 dr
B16 ndr
B16 SL dr
B16 SL ndr
C
Cx43 neg
Cx43 pos
MFI (CD11c/CD86)
C57 SL dr
B16 dr
B16 SL dr
D
IFN-γ pg/mg total protein
before SL
24h
48h

Fig. S2. Intratumoral bacterial injection increases the percentage of $Cx43^+$ DCs in lymph nodes. (**A**) *Salmonella* injected in the tumor (B16) or skin (C57) induces the increase of $Cx43^+$ dendritic cells in draining lymph nodes. C57/BL6J mice were injected with B16 cells and after 10 days were intratumorally injected with *Salmonella* (SL) or PBS as a control. After 1 or 3 days draining (dr) and not draining (ndr) lymph nodes were collected and analyzed by FACS for the presence of $CD11c^+CD4^+Cx43^+$ (left graph) and $CD11c^+CD8^+Cx43^+$ (right graph) cells. Error bars, s.d. *, $p < 0.05$. (**B-C**) Upregulation of Cx43 in dendritic cells of draining lymph nodes correlates with their activated state. The experiment was performed as in **A**, then mean fluorescence intensity (MFI) of CD86 in $CD11c^+$ cells (**B**) and the MFI of CD86 in $CD11c^+$ cells in the Cx43 negative and positive fraction (**C**) was analyzed by FACS. Error bars: s.d. *, $p < 0.05$. (**D**) Analysis by ELISA of IFN–γ in the serum of the above described mice. Error bars: s.e. *, $p < 0.05$.

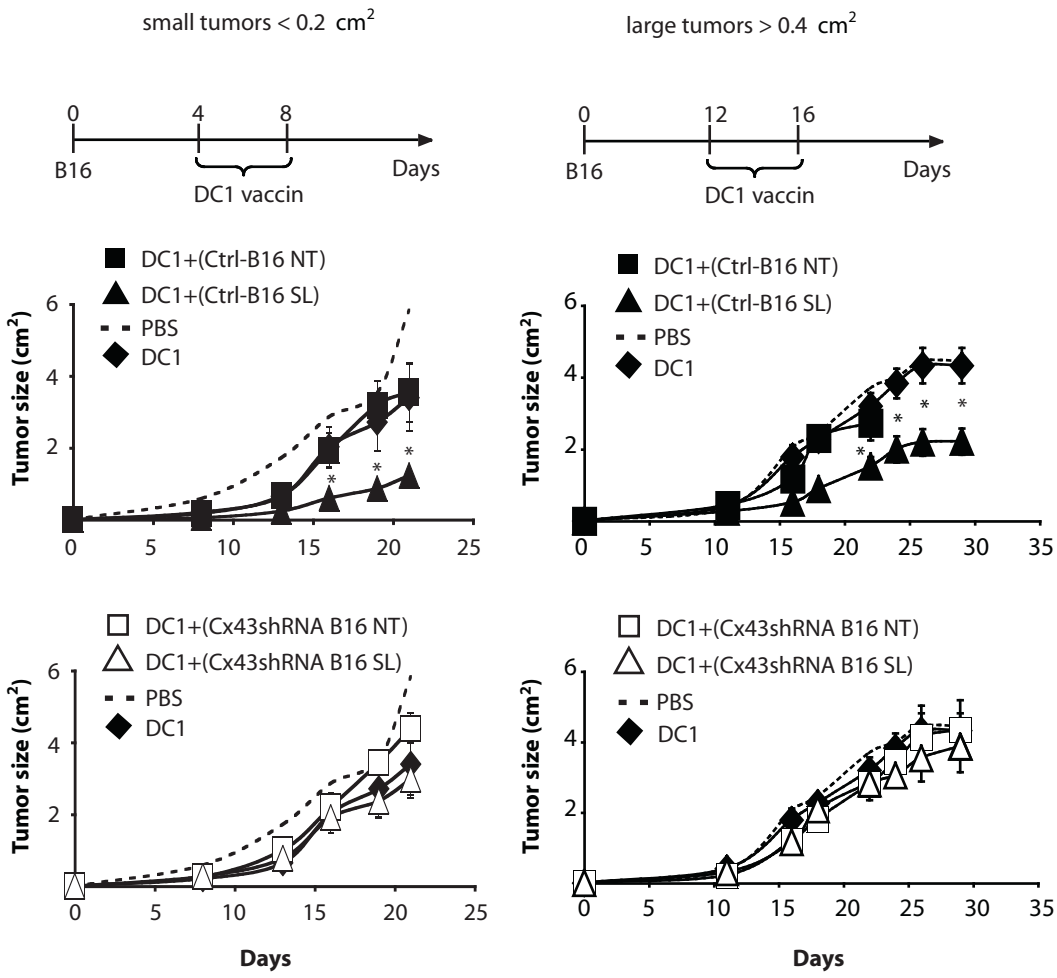
small tumors < 0.2 cm2
large tumors > 0.4 cm2
0
4
8
B16
Days
DC1 vaccin
0
12
16
B16
Days
DC1 vaccin
DC1+(Ctrl-B16 NT)
DC1+(Ctrl-B16 SL)
PBS
DC1
Tumor size (cm2)
DC1+(Cx43shRNA B16 NT)
DC1+(Cx43shRNA B16 SL)
PBS
DC1
Tumor size (cm2)
Days
Days

Fig. S3. Loading of DCs with bacteria-treated tumor cells results in efficient antitumor vaccination in a therapeutic setting. DCs were incubated with bacteria-treated or untreated B16 cells interfered or not for Cx43. Mice (n=8 per group) were injected with B16 cells and 4 and 8 days later, when the tumor was < 0.2 cm^2 (left graphs), or 12 and 16 days later when the tumor was > 0.4 cm^2, mice were vaccinated with DCs purified after loading with tumor cells. The growth of the tumors that did receive PBS as a control (PBS, dashed lines), unloaded DCs (DC1, black diamonds), DCs loaded with bacteria-treated (DC1 + B16 SL, black triangles) or untreated (DC1 + B16 NT, black squares) B16, or with bacteria-treated (DC1 + Cx43shRNAB16 SL, empty triangles) or untreated (DC1 + Cx43shRNAB16 NT, empty squares) Cx43-knocked-down B16 is shown. Error bars, s.e. *, $p < 0.05$ when comparing the growth of the tumors of mice vaccinated with DC1 + B16 SL versus DC1 + B16 NT.

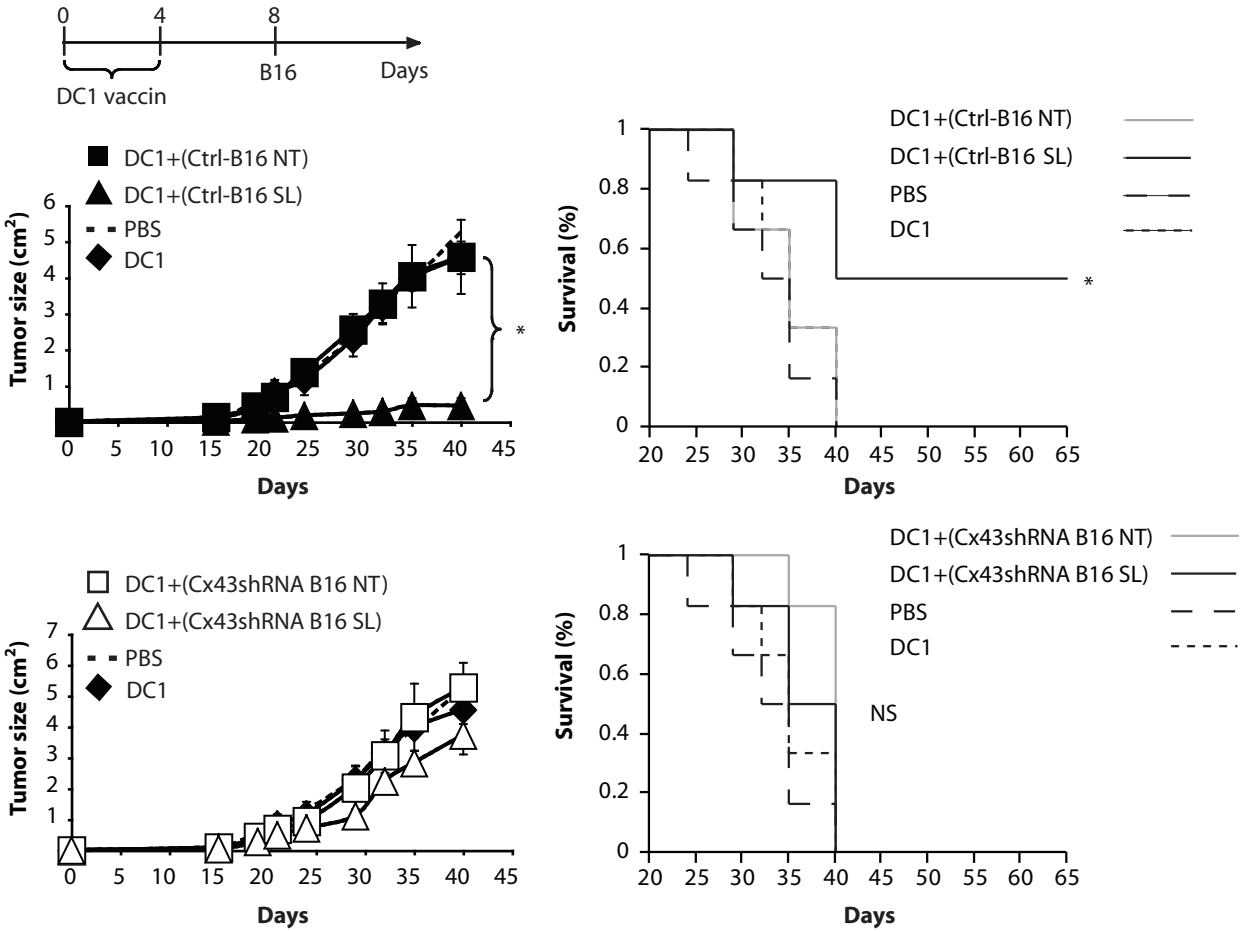
0
4
8
B16
Days
DC1 vaccin
DC1+(Ctrl-B16 NT)
DC1+(Ctrl-B16 SL)
PBS
DC1
Tumor size (cm2)
Days
*
Survival (%)
DC1+(Ctrl-B16 NT)
DC1+(Ctrl-B16 SL)
PBS
DC1
*
Days
DC1+(Cx43shRNA B16 NT)
DC1+(Cx43shRNA B16 SL)
PBS
DC1
Tumor size (cm2)
Days
Survival (%)
DC1+(Cx43shRNA B16 NT)
DC1+(Cx43shRNA B16 SL)
PBS
DC1
NS
Days

Fig. S4. Loading of DCs with bacteria-treated tumor cells results in efficient antitumor vaccination in a preventive setting. At days 0 and 4 mice (n=8 per group) were vaccinated with DCs loaded as described below, or injected with PBS as control. 8 days later, mice were injected with B16 cells. Dashed lines represent the growth of PBS treated tumor-bearing mice. Upper graphs: unloaded DCs (DC1, black diamonds), DCs incubated with *Salmonella*-treated (B16 SL, black triangles) or not (B16 NT, black squares) B16 cells. Lower graphs: DCs loaded with bacteria-treated (DC1 + Cx43shRNAB16 SL, empty triangles) or untreated (DC1 + Cx43shRNAB16 NT, empty squares) Cx43shRNAB16. Error bars, s.e. *, $p < 0.05$ when comparing the growth of the tumors of mice vaccinated with DC1 + B16 SL versus DC1 + B16 NT. NS, not significant.

Movie S1. Bacteria-induced up-regulation of Cx43 expression correlates with establishment of functional gap junctions. B16 cells were infected with *Salmonella* and after 24 hours were stained with calcein-AM (in green/white), while DCs, previously treated with LPS for 1 hour, were stained with DDAO (in red). A drop of each population was plated onto a microscope slide close to each other, and the cells were co-incubated for 1 hour. The cells were analyzed by confocal microscopy for 2 hours. The movie was generated with Imaris software.